The National Eye Institute

Annual Report

Fiscal Year 1984

THE NATIONAL EYE INSTITUTE

ANNUAL REPORT

Fiscal Year 1984

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The National Eye Institute's budget increased to over $155 million during FY 1984, permitting the Institute to support 1,067 extramural research project grants, of which 930 were individual Research Project Grants (ROIs). The Institute also supported 25 Core Grants, 35 Small Grants, 35 contracts, 222 full-time training positions through Individual and Institutional National Research Service Awards (NRSAs), and 86 intramural research projects.

During the past year scientists working at NEI have made great progress. Such advances, all of which are discussed in detail in this Annual Report, include the following:

- NEI intramural investigators have made gains in understanding the regulation of delta-crystallin gene activity and have developed a tissue culture system for studying crystallin gene promoters. Such projects have established the foundation for detailed analysis of the DNA sequences required for proper regulation of the crystallin genes.

- The roles of the enzyme aldose reductase (AR) and of AR inhibitors in diabetes have been further elucidated, suggesting that AR inhibitors might prove useful in controlling the manifestations of this disease.

- Intramural scientists have also identified an abnormality in the regulatory protein interferon-gamma and an altered expression of a cell surface antigen, HLA-DR, in retinitis pigmentosa. These investigations indicate the possibility of an underlying abnormality in biologic control mechanisms in this disease, which affects over 400,000 people in the United States.

- The effectiveness of cyclosporine, an endecapeptide with specific anti-T-cell characteristics, is being tested in children with sight-threatening ocular inflammatory disease of noninfectious origin, administered to patients with ocular cicatricial pemphigoid, and used in a randomized, double-masked study of retinitis pigmentosa patients manifesting in vitro proliferative responses to the retinal S-Ag.

- Other NEI scientists have analyzed how the frontal eye fields of monkeys control visually guided eye movements, thus providing an elegant description of sensorimotor processing in the cerebral cortex, and have established the role of the diencephalic structure, the basal ganglia, in the initiation of saccadic eye movements.

As in the past, in FY 1984 the extramural program of the NEI supported very important research, including notable projects applying new techniques in molecular biology, genetics, and immunology to vision research. Other projects have been directed toward the causes, pathogenic mechanisms, and treatment of various ocular disease processes. Fifty percent of approved research grants were funded, reflecting the NEI's continued concentration

of support on the R01 mechanism, rather than on large multiproject awards, including program projects and centers. Significant contributions have been made in a number of areas of vision research, including the following:

- A clinical trial of photocoagulation treatment with the argon laser of presumed ocular histoplasmosis syndrome (POHS) has shown that this form of treatment dramatically reduces the risk of severe visual loss in people with POHS who have active growth of new blood vessels in the macula.

- NEI-supported investigators have recently confirmed the localization of the gene or genes which predispose individuals to hereditary retinoblastoma, an ocular, tumor-producing disease, to the q14 band on chromosome 13. Patients with this sight- and life-threatening disease are also at high risk for developing nonocular tumors.

- An important first step in locating the gene locus for retinitis pigmentosa has been made through NEI-sponsored research showing that the disease locus is closely linked to a polymorphic DNA marker located in the proximal portion of the short arm of the X-chromosome. Additional research in this area may provide important information for genetic counseling of those afflicted with or having the genetic capacity to pass on this inherited disease.

- NEI-supported investigators have developed a hypothesis for the pathogenic processes which cause the retinochoriditis associated with herpes simplex virus-2 (HSV-2) infections, postulating that HSV-2-infected mononuclear cells become entrapped in the capillaries of the retina and choroid because of viral alteration of the mononuclear cell surface.

- Previously cloned murine alpha A_2 lens crystallin cDNA has been used to isolate the human alpha A_2 lens crystallin gene. This will allow complete analysis of this gene in individuals with normal lenses and those with hereditary cataracts.

- Histopathologic studies have indicated that the failure of glaucoma surgery is commonly due to the scarring over of a filtering bleb. Researchers supported by the NEI have tested 5-fluorouridine, an anti-metabolite, on experimental filtering blebs in monkeys and on humans with poor prognoses for filtering surgery. Encouraging results were obtained in both groups indicating that inhibition of fibroblast proliferation might improve the success rate for this type of surgery.

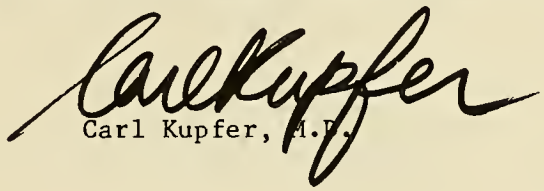
- Tests for assessing visual function behind ocular opacities have been developed to aid surgeons in predicting whether or not vision is likely to be restored by cataract removal. Application of these tests to selected patients has had good predictive value of visual function after cataract surgery.

- o NEI-supported research into the nature of the visual sensory deficit in amblyopia has indicated that spatial abnormalities seem to be the primary deficit in strabismic amblyopia whereas impaired resolution may be the primary deficit in anisometric amblyopia.

In the past year a multicenter clinical trial of the aldose reductase inhibitor sorbinil has continued with NEI's scientific leadership and Pfizer Laboratories' funding.

NEI's established collaborative studies on nutritional blindness with India's National Institute of Nutrition in Hyderabad are continuing. Under the US-Indo Science and Technology Initiative, the scope of NEI's collaboration has expanded to include a case control study of risk factors in senile cataract at the Dr. Rajendra Prasad Ophthalmic Center in New Delhi and a clinical trial in the treatment of Eales' disease and its etiology at the Aravind Eye Hospital and Madurai Kamaraj University.

The reports of all the NEI branches and offices which follow expound on these and other activities and accomplishments of the NEI in FY 1984.



Carl Kupfer, M.D.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS
Ronald G. Geller, Ph.D.

In keeping with the Institute's first priority, support for investigator-initiated individual research projects (R01, R23, R43, & R44), 969 such awards were made in FY 1984 covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. This represents about 87 percent of the NEI extramural budget. The following program reports highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1984.

For FY 1984, the National Eye Institute received an appropriation of $155,131,000--an increase of $13,230,000 over the previous year's appropriation. Of the $155,131,000, a total of $132,903,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Extramural and Collaborative Programs
Research, Training, and Contract Funding
(Dollars in Thousands)

Research Grants	$121,992
Research Training Awards	3,900
Research Contracts	6,220
Small Business Innovative Research Awards	791
Total	$132,903

These funds were distributed among the Institute's five research programs as follows:

Extramural and Collaborative Programs
Research, Training, and Contract Funding
(Dollars in Thousands)

Retinal and Choroidal Diseases	$ 57,997
Corneal Diseases	20,947
Cataract	11,676
Glaucoma	12,997
Strabismus, Amblyopia, and Visual Processing	29,286
Total	$132,903

The Institute was able to fund 51 percent of all applications. Historical data are given below:

Grant Application Rate*

	Received & Reviewed	Recommended For Approval	Approved & Funded	% Funded of All Approved Applications
FY 1978	681	562	343	61
FY 1979	579	495	308	62
FY 1980	516	432	225	52
FY 1981	636	606	309	51
FY 1982	629	564	273	48
FY 1983	623	524	247	47
FY 1984	666	588	301	51

*(For R01s and R23s)

The distribution of awards (for R01s and R23s) between competing and non-competing research grant applications was as follows:

	FY 1982 Number of Grants	FY 1983 Number of Grants	FY 1984 Number of Grants
Prior Year Commitments	680	717	660
New Research Awards	135	129	136
Renewal Awards	140	112	165
	955	958	961

The Institute's research grants comprised the following categories:

FY 1983 Research Grants by Mechanism
(Dollars in Thousand)

	Number	Total Awarded
Research Project Grants (R01, R23)	961	$114,816
Core Grants (P30)	25	4,153
Specialized Clinical Research Center Grants (P50)	1	869
Research Career Development Award (K04)	17	685
Other Research and Research-related Grants (U09, U50, R13, S06)	20	727
Small Grants (R03)	35	742
Small Business Grants (R43, R44)	8	791
Total Research Grants	1,067	$122,783

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences, and insights necessary for them to embark successfully on a career in vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering, and biomathematics.

A total of $3,900,000 was available for support of vision research training in FY 1984. The individual NRSA fellowship awards accounted for $1,516,000, or 38.9 percent of available training funds. The institutional NRSA training awards accounted for $2,384,000, or 61.1 percent of the program. A summary of the training program for FY 1984 follows:

Vision Research Training FY 1984
(Dollars in Thousands)

	Institutional (NRSA T32)				Institutional (NRSA F32)		
	No. of Inst. Awards	Pre-Doctoral	Post-Doctoral	Amount	No. of Ind. Awards	Amount	Total (T & F)
Retinal and Choroidal Diseases	11	11	38	873*	29	522	1,395
Corneal Diseases	7	7	26	706	16	312	1,018
Cataract	0	0	0	0	3	54	54
Glaucoma	2	0	9	193	3	51	244
Strabismus, Amblyopia, and Visual Processing	12	19	27	612	34	577	1,189
TOTALS	32	37	100	2,384	85	1,516	3,900

* $11,000 of this total represents NEI's co-funding of two T35's (short-term training program) under the auspices of the NIGMS for eight predoctoral positions.

RETINAL AND CHOROIDAL DISEASES

INTRODUCTION

The retina is the delicate, multilayered, light-sensitive membrane that lines the inside of the back of the eye. Contained within this stratified tissue are a mosaic of photoreceptor cells (the rods and cones) and an exquisitely organized system of other nerve cells and associated elements that contribute to its normal function. Images of the external world that are formed on the retina by the eye's optical elements are converted to electrical signals, encoded into a visual message, and transmitted to the brain via the optic nerve.

The retinal neurons are highly differentiated cells which, if irreparably damaged, are incapable of being replaced by cellular division. They depend for their survival on a carefully controlled environment and a continuous supply of oxygen and nutrients derived from two systems of blood vessels, one intrinsic to the retina, the other in the highly vascular choroid. Any damage to the retina, interruption in its blood supply, or injury to the tissues with which it interacts leads to loss of vision. Unfortunately, the retina is susceptible to injury in a remarkable variety of ways, ranging from hereditary degenerative disorders to diseases associated with infections, and from damage by toxic agents to visual loss resulting from retinal detachment, diabetes, and circulatory failure. Each may have devastating consequences for vision and the conduct of a normal, productive life.

Over 750,000 Americans with a retinal disorder suffer from visual impairment that is so severe they are unable to read ordinary newsprint, even with glasses. Of these, 200,000 are legally blind, making this group of diseases the leading cause of blindness in the United States. In fact, one of these diseases, diabetic retinopathy, is the leading cause of new cases of blindness in adults under age 65, and another, age-related macular degeneration, is the leading cause of new cases of blindness in people aged 65 and older. Every year, an additional 19,000 Americans become blind from retinal and choroidal disorders.

PROGRAM STRUCTURE

During fiscal year 1984 the Retinal and Choroidal Diseases Branch awarded more than 408 grants to individual investigators in support of research in this area. The distribution of these grants by subprogram is presented in the following table. Highlights of research accomplishments during the past year for several of these areas are presented below.

Subprogram/Area	FY 1984 Grants*
VASCULAR, INFLAMMATORY, AND NEOPLASTIC DISORDERS OF THE RETINA AND CHOROID	
Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities	49
Inflammatory Disorders	16
Tumors	19
DEGENERATIVE DISORDERS OF THE RETINA	
Developmental and Hereditary Disorders	33
Macular Degeneration	24
Retinal Detachment and Vitreous Disorders	18
Toxic and Environmental Disorders	3
FUNDAMENTAL PROCESSES AND RETINAL DISORDERS	
Retinal Pigment Epithelium	30
Photoreceptors, Visual Pigments, and Phototransduction	119
Retinal Organization, Neurotransmission, and Adaptation	85
Glial Cells and the Retinal Microenvironment	4
Rescue and Regeneration of Neurons in the Optic Nerve and Retina	3
RELATED AREAS OF RESEARCH OPPORTUNITY AND NEED	
Noninvasive Techniques in the Study of Retinal Disorders	5
Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models	0**
TOTAL	408***

* Includes R01, R03, R23, P50, and K04 mechanisms (when applicable).
** Related grants counted elsewhere within Retinal and Choroidal Diseases Subprograms.
*** Totals include 11 Small Grants for Pilot Projects (R03).

RECENT ACCOMPLISHMENTS

Successful Clinical Trials Results

The Retinal and Choroidal Diseases Branch supports a large program of clinical trials. Included are studies of the efficacy and safety of various treatments for retinal disorders including diabetic retinopathy, age-related macular degeneration, presumed ocular histoplasmosis syndrome (POHS), branch retinal vein occlusion, retinitis pigmentosa, and retinopathy of prematurity. During FY 1984, results of two large-scale multicenter studies were announced.

Ocular Histoplasmosis. About 4 percent of the many people in the United States who react positively to the histoplasmin skin test, have scars (histo spots) in the macular region of their retinas. Presumed ocular histoplasmosis syndrome, which is marked by subretinal neovascularization followed by retinal degeneration, is believed to begin with the growth of new vessels from the choroid into the retina in the region of one of these histo spots. Often, the process continues until most of the macula, and the fovea, have been destroyed. Yet, in many cases the process is spontaneously arrested.

Some ophthalmologists have believed for years that photocoagulation treatment with the argon laser is helpful in preventing severe visual loss in people who have neovascular POHS. However, others have been reluctant to offer laser treatment because there has been no proof of its effectiveness in preserving vision, and because its use causes some destruction of healthy tissue in the area treated by the laser beam.

To determine whether laser treatment can be of value to people who are in imminent danger of losing vision from POHS, the Retinal and Choroidal Diseases Branch has been supporting the nationwide Ocular Histoplasmosis Trial, at 12 medical centers across the United States. The 245 POHS patients in the study were chosen because they showed active growth of new vessels in the macula of at least one eye but had not yet lost their foveal vision in that eye.

Results from the study indicated that photocoagulation of new blood vessels in patients with POHS dramatically reduced their risk of severe visual loss. Forty-three percent of the untreated eyes lost most of their central vision, while only 19 percent of the eyes that had laser treatment at the beginning of the study experienced such a major loss in visual function. It was therefore concluded that argon laser photocoagulation is effective in reducing the risk of severe visual loss in people with POHS who have new blood vessels growing into the macula.[1]

Branch Vein Occlusion. The Branch Vein Occlusion Study is a five-center, randomized, controlled clinical trial designed to answer three questions regarding argon laser treatment of complications of branch vein occlusions: 1) Can photocoagulation prevent the development of neovascularization? 2) Can photocoagulation prevent vitreous hemorrhage? and 3) Can photocoagulation improve visual acuity in eyes with reduced acuity due to

branch vein- associated macula edema? In September 1983, the Branch Vein Occlusion Study Group announced a positive finding with respect to the third question.[2]

One hundred thirty-nine eyes with macular edema and visual loss associated with branch vein occlusion were assigned randomly to either an argon laser treated group or to an untreated control group. After a mean follow-up of 3.1 years, a significantly greater proportion of the treated patients were found to have improved vision, and were more likely to sustain the improvement in their vision. As a result, the study group has recommended that patients with macular edema associated with branch vein occlusion receive photocoagulation treatment. Answers to the remaining two questions concerning treatment of branch vein occlusion complications should be available within the next two years.

NEI Workshop: Molecular Genetic Approaches to the Study of the Retina

Vision Research--A National Plan: 1983-1987 makes frequent reference to the opportunity and need for an increased application of recombinant DNA concepts and technologies to research on the retina, the retinal pigment epithelium, and the inherited retinal degenerations. Related to this is the recognized need to attract to vision research new investigators from the field of molecular genetics. The National Advisory Eye Council enthusiastically approved the concept for a workshop to help meet these objectives.

Accordingly, the NEI organized and sponsored a workshop entitled "Molecular Genetic Approaches to the Study of the Retina," held February 16-17, 1984, in Chantilly, Virginia. Drs. Richard N. Lolley, UCLA, and H. Gobind Khorana, MIT, were the co-chairmen for the event. Molecular geneticists, neuroscientists, cell and developmental biologists, and vision scientists participated. A number of students, fellows, and junior investigators from leading molecular genetics laboratories were invited and took part in the discussion.

Dr. Khorana provided an overview of molecular genetic strategies and techniques as they relate to the study of the retina. Drs. Paul A. Hargrave (Southern Illinois University), Meredith L. Applebury (Purdue University), Daniel D. Oprian (MIT), Jeremy Nathans (Stanford University), and Toshimichi Shinohara (NEI) led a discussion of molecular genetic studies of the visual pigments.

The use of recombinant DNA and hybridoma techniques in the study of the "phosphodiesterase activation cascade" was the subject of a second session led by Drs. Lubert Stryer (Stanford University), M. Deric Bownds (University of Wisconsin), Bernard K. Fung (University of Rochester), Hurley (California Institute of Technology), Yatsunami (MIT), and Richard N. Lolley (UCLA).

Dr. A. Linn Murphree (Children's Hospital of Los Angeles) presented evidence that the retinoblastoma-predisposing allele is recessive at the cellular level. Dr. Webster K. Cavenee (University of Cincinnati) documented how various chromosomal mechanisms can operate to produce this homozygosity. Dr. Alfred Gilbert (Mount Sinai School of Medicine) described his studies which indicate that DNA amplification of the oncogene n-myc may be involved in the development of at least some retinoblastomas. Dr. Thaddeus P. Dryja

(Howe Laboratory of Ophthalmology) presented recent work which may help determine the meiotic recombination distance between the retinoblastoma locus and the loci of various chromosome 13 DNA probes.

There was considerable discussion, led by Drs. David E. Housman (MIT) and Eliot L. Berson (Harvard), of how recombinant DNA tools could be useful in the study of inherited retinal degenerations. Particular emphasis was placed on the exciting opportunities for genetic linkage studies of retinitis pigmentosa using recombinant DNA probes (restriction fragment length polymorphisms).

Presentations in a final mini-workshop were made by Drs. Matthew M. LaVail (UCSF), C. David B. Bridges (Baylor), Mary Beth Burnside (UC, Berkeley), Michael O. Hall (UCLA), and David Valle (Johns Hopkins). These investigators reviewed recent research on the known interactions between photoreceptor cells and the retinal pigment epithelium and the possible role of these interactions in the etiology of inherited retinal degenerations. Much of the discussion was directed at identifying specific problems that are being or should be approached using molecular genetic techniques. Special emphasis was placed on the function, utilization, and transport of retinoids in the visual cycle; on the role of Ca^{2+} and cAMP in regulating motile processes in the retina and retinal pigment epithelium; on the molecular basis for the defective phagocytosis in the RCS rat; and on studies of gyrate atrophy of the retina and choroid.

Throughout the workshop the theme was expressed that rapid progress in the application of molecular genetic techniques to problems in retinal research will critically depend on the free exchange of ideas, information, and materials.

Retinoblastoma

Retinoblastoma occurs in hereditary and nonhereditary forms. The hereditary form (35 to 40 percent of all cases) is inherited as an autosomal dominant trait with a penetrance of about 90 percent.[3] Most (85 to 90 percent) patients with the hereditary form of retinoblastoma develop tumors in both eyes. These patients, in addition, are at high risk for subsequently developing nonocular tumors, especially pinealoblastoma and osteogenic sarcoma.[4] Patients with unilateral, nonhereditary retinoblastoma are not at high risk for developing second primary tumors. It is, therefore, important to detect retinoblastoma early and to distinguish the hereditary from the nonhereditary form.

Recent studies of retinoblastoma are utilizing gene probes and other tools drawn from recombinant DNA technology and cytogenetics. NEI-supported investigators have been major contributors to a pioneering research effort that may set the stage for new approaches to genetic counseling and for eventually isolating and characterizing the gene(s) whose defect predisposes to retinoblastoma. Molecular genetic studies of retinoblastoma were identified as an NEI Program Development Priority in Vision Research--A National Plan: 1983-1987 (Volume Two/Part 1, page 50).

A small minority of hereditary retinoblastoma patients have a deletion on chromosome 13 which always involves band q14. This observation led to the hypothesis that somewhere in this region is the gene or genes predisposing one to retinoblastoma. Sparkes, Murphree, and their collaborators recently confirmed this localization by the demonstration of very tight linkage between the loci for hereditary retinoblastoma and the polymorphic enzyme esterase D, previously assigned to chromosomal region 13q14.[5]

Recombinant DNA technology has been used to generate new DNA markers (termed restriction fragment length polymorphisms) that can be used to look for genetic differences. (See extended discussion below.) These DNA markers are generated by the action of restriction endonucleases which recognize specific DNA sequences and cut at specific sites. Thus, differences in DNA sequences between individuals can be used directly as genetic markers without knowledge of the identity of the proteins coded for by the genes. Exciting results using this type of approach were recently reported by Cavenee and his colleagues.[6] The data give strong support to the hypothesis that inherited retinoblastoma results from the development of homozygosity for a mutant allele on chromosome 13. This approach also enables one to determine from a number of possible mechanisms the different ways that homozygosity might come about. Evidence has been marshaled to suggest that retinoblastoma may be a model for a new class of recessive human cancer genes.[6,7] Efforts are now underway in several laboratories to determine if available cytogenetic and DNA markers are linked sufficiently closely to the retinoblastoma locus to be useful in genetic counseling.

Molecular Genetic Linkage Studies of Retinitis Pigmentosa

Neither a specific biochemical defect nor an exact gene locus has been found for any of the major forms of inherited retinitis pigmentosa (RP). Recombinant DNA technology, however, has led to a new approach to the study of RP. The new approach involves the use of cDNA probes of known chromosomal loci to detect DNA sequence polymorphisms (DNA markers) linked to RP. The DNA markers are generated by the action of restriction endonucleases, enzymes which recognize specific DNA sequences and cut DNA into fragments. The size of these fragments will vary among individuals depending on what specific DNA sequences have been inherited. Individuals inherit a variable assortment of these DNA markers, just as they inherit a variable assortment of physical and biochemical traits. These DNA markers can be easily separated by electrophoresis and identified by reference to cDNA probes of known chromosomal loci. The aim is to determine whether or not a specific DNA marker is being transmitted together with the RP trait. By sequential analyses of this type, it should be possible to map the RP gene with respect to the known DNA marker loci. Note that this new approach does not require any prior knowledge of the exact DNA sequence of the DNA marker or of the biochemical products coded by this stretch of DNA.

A number of investigators in this country and abroad have begun genetic linkage studies of RP using this new approach. The field has been spurred on by a significant advance in the study of X-linked RP. Wright, Bhattacharya, Bird, and colleagues have reported that the disease locus is closely linked to a polymorphic DNA marker that maps to the proximal portion of the short arm of the X-chromosome.[8] This is an important first step for eventually locating the gene locus for X-linked RP. At present, this particular DNA

marker is still very limited in its value for genetic counseling. Additional families must be studied; additional cDNA probes and DNA markers must be tested.

An even greater increase in research activity is expected as investigators expand the work on X-linked RP, and extend the analyses to the autosomal dominant and recessive forms of the disease.

Spectral Analysis of Single Cones

The photoreceptor cells of the retina are biological transducers that convert light energy into neural signals. There are two kinds of photoreceptor cells in the retina: rods and cones. Rods contain the photopigment rhodopsin which is the protein opsin bound to the chromophore vitamin A or 11-cis retinal. Three types of cones have been identified in mammalian species and are named according to their spectral absorption characteristics: red sensitive (610 nm), green-sensitive (540 nm), and blue-sensitive (440 nm). In the case of the cone pigments, the chromophore, 11-cis retinal, is the same as the one that is bound to rhodopsin. Thus, cone pigments differ from rhodopsin in the nature of the protein component. Up until now, all the evidence for the existence of separate cone types has been indirect and has been provided by psychophysical color matching data and absorbance spectra of cones using microspectrophotometry. Recently, Nunn et al.[9] documented direct evidence for the identity of separate photopigment-containing cells. These investigators obtained the electrophysiological action spectrum from individual cones in the monkey retina using the technique of suction electrode recording whereby individual photoreceptors are gently pulled into a recording micropipette. Electrical responses from individual middle- and long-wavelength cones were recorded which corresponded to the green and yellow-green portion of the light spectrum. With this technique it will now be possible to answer some interesting questions on, for example, adaptation using both microspectrophotometric and electrophysiological measurements on single photoreceptors. An important implication of this work is that some basic information on cone function can now be obtained from an animal thought to have cone pigments very similar to those in man.

Molecular Biology of Genes Coding for Visual Pigment Proteins

Breakthroughs are also being made in the isolation and characterization of the genes which code for the photopigments. Nathans and Hogness are attempting to use the coding sequences in the bovine opsin gene as a hybridization probe to identify and isolate human opsin genes.[10] To this end a cloned cDNA segment which contains sequences from the bovine rhodopsin gene has been used to screen a library of cloned human DNA sequences. One of the nucleotide sequences contains a 7.2 kilobase fragment which appears to have a gene structure almost identical to bovine opsin. The deduced amino acid sequence consists of 348 residues and is almost completely homologous with the known sequence of bovine opsin. Additional evidence that this gene encodes human rod opsin comes from the fact that the codon sequence is interrupted by four introns at positions identical to those for the bovine opsin gene. In addition to breakthroughs in identification of the rod opsin gene it appears that genes coding for the cone opsins may be nearing identification. The reason for this optimism is that some segments of the rod opsin gene appear to have sequences which are identical to portions of the genes coding for the cone

opsins. Some weakly hybridizing cDNA probes have been found to correspond to fragments from total human DNA. These may represent genes encoding one or more of the human cone opsins. In work carried out by Applebury, a full-length clone encoding bovine opsin has been completely sequenced.[11] Analysis shows that the deduced amino acid sequence of the opsin-coding region is consistent with the sequence obtained by direct analytical methods. There does not appear to be a leader sequence in this protein as is found for some other transmembrane proteins. Vision Research--A National Plan: 1983-1987 specifically calls for the isolation of "the gene that codes for rhodopsin" and an examination of the relationship between rod and cone genes and the visual pigments whose expression they control.

Glial Cells and the Retinal Microenvironment

One of the Program Development Priorities in Vision Research--A National Plan: 1983-1987 (Vol. 2, Part 1) is to encourage research which seeks to quantitate extracellular and intracellular activities of ions in the retina and to study the mechanisms of ion buffering. A recent accomplishment has been achieved in just this area:

Glial (Muller) cells traverse the several layers of the retina in a radial direction from the internal to the external limiting membrane, projecting beyond the latter to the outer nuclear layer where it envelops the perikarya of the rods and cones in honeycomb-like fibrillar "baskets." Because of its close contact with most retinal cell types as well as the vitreous, the Muller cell is in a position to influence the microenvironment of neighboring neurons. Muller cells have long been thought to play an important role in the generation of some components of the electroretinogram (ERG). The reason for this is that the Muller cell is almost exclusively permeable to potassium ions and the "b" wave of the ERG has been thought to originate from extracellular potassium efflux following neural activity. One of the general functions of glial cells is to take up potassium from regions around neurons where it builds up as a result of cellular electrical activity. This forms the basis for the "spatial buffer" concept of glial cell function. A new role for the Muller cell has been proposed by Newman.[12] His studies suggest that the specialized endfeet of Muller cells, located adjacent to the vitreous, function to transfer potassium into and out of the vitreous which appears to act as a large reservoir for potassium ions. Dr. Newman's research indicates that Muller cells may function less by moving potassium ions between retinal layers and more by transport of ions into and out of the vitreous. The importance of this work lies in the fact that Muller cells are believed to contribute to generation of extracellular field potentials which are reflected in the wave-pattern of the ERG. Analysis of the origin of the components of the ERG will allow more specific identification of the retinal cell types which may become injured or diseased. This information could help lead to earlier detection of a retinal lesion and more timely treatment.

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CORNEAL DISEASES

INTRODUCTION

The cornea is the clear, essentially colorless avascular tissue at the front of the eye which function as a powerful lens. The cornea contains numerous sensory nerve endings that subserve touch and pain. There are no lymph vessels or other channels for bulk fluid flow contained in this structure. The interface between the corneal tear film and the ambient atmosphere provides roughly two thirds of the refractive power of the human eye. If injured, the outermost layer of the cornea, the epithelium, is capable of regeneration and rapid healing without scarring. However, injury to the deeper corneal layers often produces an opacity.

Corneal injury and disease often cause intense physical discomfort; sizable expenditures for physician care, medication, and possibly hospitalization; and days lost from work. Every year, more than 2 million cases of corneal disorders and at least 1.7 million injuries to the cornea are recorded.

Corneal problems constitute 62 percent of the total incidence of all acute and chronic afflictions of the eye, accounting for more than 100,000 hospital days and $12 million in surgical costs annually. Aside from refractive errors, injuries and disease of the cornea require 10 million annual office visits, or one-third of all visits for medical and surgical eye treatment, and thus consume a major portion of the eye-care dollar.

PROGRAM STRUCTURE

The Corneal Diseases program of the National Eye Institute is divided into the following five subprograms which reflect the breadth and diversity of ongoing research in this field. These categories are interrelated in a variety of ways so that research accomplishments in one area may have a major influence on others.

Subprogram	FY 1984 Grants*
1. External Ocular Infections and Inflammatory Diseases	48
2. Ocular Surface Problems	42
3. Refractive Problems and Contact Lenses	21
4. Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Diseases	38
5. Corneal Transplantation and Wound Healing	25
TOTAL	174

* Includes R01, R03, R23, and, K04, mechanisms (where applicable)

RECENT ACCOMPLISHMENTS

External Ocular Infections and Inflammatory Diseases

Herpes simplex. Viral keratitis and other keratopathies are debilitating diseases of major importance in the United States and throughout the world. Keratitis, which results from infection by herpes simplex virus (HSV), has an incidence of approximately 500,000 cases a year in this country, and there is a high level of morbidity associated with such attacks.[1,2] After one ocular infection with type 1 herpes simplex virus, the chances are almost 50 percent that the infected individual will have another attack within 2 years;[3] the risk increases if the patient has had two or more attacks.

The ocular damage caused by herpes infection is largely due to its chronic recurrent pattern; more permanent damage occurs with each subsequent attack. Following the initial infection, HSV may become inactive or latent in neuronal tissue, only to reactivate unpredictably at some later time, causing recurrence of the disease. In its latent stage the virus is not susceptible to any drugs which are currently available.

Genital herpes is the second most common venereal disease in this country and is caused primarily by type 2 virus. Herpes infection of the maternal reproductive system infects newborn infants in as many 1 out of 7000 births,[4] and this incidence rate is expected to increase rapidly due to the recent explosive increase in genital infections produced by HSV-2 in the United States.[5]

The eye can be the primary and only site of involvement in neonatal herpes, but it also can commonly be associated with both disseminated and localized forms of the disease. Approximately 10 percent of neonates with disseminated infection and one-third of those with localized infections have ocular involvement.[6] In each of these groups, one or more combinations of conjunctivitis keratitis, chorioretinitis, and cataracts can occur.

A possible explanation for the pathogenic processes which cause the development of the retinochoroiditis associated with HSV-2 infections in neonates comes from the newborn rabbit studies of Oh and coworkers.[7] Following systemic infection, it is believed that HSV-2 induces Fc receptors [antigenic determinants on heavy chains of immunoglobulin G (IgG)] for normal immunoglobulin G on mononuclear cells present in the blood. The binding of IgG to the Fc receptors is thought then to change the nature of the cell surface of the HSV-infected mononuclear cells and lead to the entrapment of these cells in the capillaries of the retina and choroid. These entrapped HSV-containing cells initiate infection at the site where these cells are lodged and thus produce a retinochoroiditis. These investigators are now further characterizing the Fc receptor sites and are attempting to determine which subpopulation of mononuclear cells is the primary carrier for the virus.

In another study, Oakes and associates have observed that mice immunosuppressed with 450R whole body irradiation develop a fatal viral encephalitis 10-12 days after inoculation of the cornea with herpes simplex virus type I.[8] Intravenous transfer of $2\text{-}3\text{x}10^7$ virus-sensitized spleen cells derived from HSV-infected mice 24 hours before virus challenge led to resolution of the infection in the central nervous system, although it did not prevent virus spreading from the eye to the brain. If the virus-sensitized cells were pretreated with a monoclonal antibody directed against the Lyt-1^+ membrane antigen on the lymphocytes, the protective effect was found to be either significantly reduced or completely abolished. These results suggest that the Lyt-1^+ subset of T cell lymphocytes which mediate delayed-type hypersensitivity and/or antibody synthesis play a predominant role in promoting recovery from HSV-1 ocular-initiated infections in mice. Similar studies need to be done with the HSV-2 virus in this animal in order to determine if the same protective mechanism is operative.

Ocular Surface Problems

Tear Film and Its Abnormalities. The tear film on the cornea's surface helps protect it from injury, infection, and the environment and also serves as a major element in light refraction. This fluid layer is responsible for maintaining the health of the cornea and conjunctiva which it bathes. Numerous diseases adversely affect the tear film, causing conditions that, in turn, affect the cornea and conjunctiva. Tear abnormalities may occur as primary and isolated manifestations of eye disease, or may accompany other ocular or systemic disease. Adverse effects of tear film abnormalities range from persistent eye irritation to severe discomfort and blindness. Although exact figures are not available, tear film disturbances probably account for eye symptoms in millions of Americans.

Vitamin A as free retinol has recently been detected in both rabbit and human tears by Ubels and MacRae[9] through analysis on high-pressure liquid chromatography. Tear samples (volume 8-20 ul) were collected from normal, vitamin A-deficient, and pair-fed control rabbits. Although a chromatographic peak for retinol was noted from tears obtained from normal and pair-fed rabbits, no comparable peak was observed in vitamin A-deficient rabbits in stage 1 or 4 xerophthalmia. It was concluded from this study that the vitamin A present in normal tears was not present in a form that was bound to retinol-binding protein. Retinol levels were found to average to 60 $\pm$ 10mg/ml ($0.2\text{x}10^{-6}$M) in normal rabbits. Retinol was also detectable at a concentration of $0.5\text{x}10^{-7}$M in tear samples (volume 20-90 ul) collected from 10 human volunteers.

This study shows that the tear film is an important source of vitamin A for the ocular surface and suggests that impaired delivery of vitamin A to the cornea may be a factor in dry-eye syndrome. Such investigations also establish the rationale for the treatment of corneal disease with topical retinoids.

Ocular Surface Disorders

The ocular surface has been defined as the epithelium and underlying tissues which line the back of the lids, cover the globe, and extend over the cornea. With the exception of the cornea, the tissues underlying the

epithelial layer are vascularized and contain large numbers of inflammatory and immunologic cells. Consideration of the corneal and conjunctival surface as parts of a single sheet of tissue has permitted a new approach to the study of surface disease, acknowledging the close relationship and interactions between these regions. The surface of the cornea is particularly prone to foreign body injuries and superficial ocular infections which necessitate many visits to ophthalmologists' offices and hospital emergency rooms. Furthermore, many surface defects, such as those caused by chemical burns, heal very slowly and frequently result in bilateral blindness.

Zieske and Gipson,[10,11] through the use of an epithelial wound-healing model in rats, have demonstrated the presence of two proteins, 70K and 110K, on the cell surfaces of migrating wounded corneal epithelium that were not present on normal stratified epithelium. In addition, an overall large increase in protein and glycoprotein synthesis has been noted in the epi-thelial layer during wound healing by employment of radiolabeling and SDS gel electrophoretic techniques on sheets of derived epithelial cells.

The 70K protein has been tentatively identified as a laminin receptor by virtue of its reactivity with a monoclonal antibody which is specific for the laminin receptor. Further characterization of the protein is necessary to determine whether this macromolecule plays a role in the attachment of the epithelial sheet to basement membrane during wound healing.

Refractive Problems and Contact Lenses

Modification of Refractive Error. Nearly 100 million people in the United States have refractive errors--nearsightnessness, farsightedness, or astigmatism--that require correction, usually by eyeglasses and/or contact lenses. About 12-15 million of these people wear contact lenses to correct their refractive errors.

Orthokeratology is a procedure which utilizes specially designed contact lenses in an attempt to modify the curvature of the cornea and thereby reduce permanently the refractive error associated with myopia. A recently completed National Eye Institute-supported study of orthokeratology--a single center, randomized controlled clinical trial--found that only about 1 to 1.25 diopters of change could be induced by this technique and that the observed change produced was not permanent unless the contact lens was worn continuously.[12,13]

The lack of persistence in retention of the shape of the cornea through use of contact lenses has been ascribed either to high elasticity of the ocular connective tissue or to some other memory mechanism. This phenomenon may represent an inherent characteristic of the cornea, and such an observation may have relevance to corneal changes observed following surgical attempts to reduce refractive error.[14]

Binder and his coworkers have employed the use of intrastromal hydrogel lenses in baboons for a study of surgical correction of aphakia.[15] The lenses were treated as if they were human donor corneas using currently practiced keratophakia procedures. The steps included microkeratome

resection of the recipient cornea, staining of the hydrogel lenses before cryolathing, and, in many instances, lathing according to a modified program after which the derived lenticules were inserted into the previously dissected recipient lamellar bed. Although no evidence of an inflammatory reaction to the hydrogel lenses was observed in the recipient corneas when they were examined histologically, activated keratocytes, phagocytic keratocytes, and macrophages were noted at the hydrogel stromal interfaces.

In subsequent studies,[16] the use of similar-shaped factory-lathed hydrogel lenticules in the laboratory animal investigations were found to cause a significant decrease in the number of phagocytic keratocyte and macrophage cell types. However, further long-term studies will be needed to evaluate whether these implants will significantly reduce the keratocyte reaction.

Another technique to reduce corneal curvature in nearsighted patients, radial keratotomy, was introduced by Fyodorov in 1974.[17] The procedure involves making 8 or 16 radial incisions in the cornea to approximately 80 to 90 percent of its depth, as one would outline a pie for cutting, but sparing the central 25 percent of the cornea which is known to be essential for vision. This operation results in a flattening of the central cornea, which improves the patient's distance vision without glasses.

The National Eye Institute responded in 1980 to growing scientific and public concerns about the long-term safety and efficacy of radial keratotomy by providing support for a scientific evaluation of this clinical procedure This support took the form of a 9 multicenter clinical trial of the 8-cut surgical procedure. Although results for the first year of evaluating radio keratotomy in nearly 500 patients will be presented at the November 1984 meeting of the American Academy of Ophthalmology, a study of the psychosocial characteristics of candidates presenting themselves for radial keratotomy in this trial has already been published by Bourque and associates.[18] Questionnaire data collected on patients in the clinical trial were compared with a similar group of myopic persons studied during the Rand Health Insurance Experiment. The clinical trial patients were found to be predominately young, white myopes who disliked being dependent on corrective lenses but perceived themselves as being more visually impaired than did comparable Rand study myopes. Most of the female subjects and a plurality of the male subjects had previously tried contact lenses and quit wearing them mainly because the use of such lenses was either inconvenient or bothersome. No evidence existed that the clinical trial subjects were either psychologically or socially deviant. Both male and female subjects expressed a fear of being without vision and cited impatience with the lenses as their major motivation for wanting surgery.

Yamaguchi and coworkers[19] have recently evaluated the extent of endothelial damage produced in monkeys after anterior radial keratotomy performed with a diamond blade. Although endothelial degeneration was seen histologically in these animals after surgery (approximately 8 percent cell loss), epithelial edema was largely absent and few edematous endothelial cells were visualized. These results were in contrast to the authors' previous findings in which a stainless steel blade was employed in the surgery and more extensive endothelial degeneration and epithelial edema was noted.[20] The fear was expressed by the investigators that the

cumulative loss of cells due to surgical trauma, combined with continuous damage-related losses and later age-related reduction in cell numbers, could produce a corneal decompensation in some patients years after receiving radial keratotomy.

Corneal Transplantation

Keratoplasty represents the oldest and most successful of the solid tissue transplants performed on humans. This surgery has been employed regularly since the 1940s for the purpose of restoring vision to eyes with clouded corneas, and it is estimated that 22,000 to 23,000 corneas are transplanted annually in the United States.[21]

The greater success rate achieved with corneal transplantation compared with other organ transplants has been attributed to the fact that the cornea is a site of "immunological privilege." Although corneal cells are known to possess the antigens of the major histocompatability complex responsible for allograft rejection in other tissues (i.e., HLA the antigens),[22] normal avascularity of the cornea has been cited as the reason for the relative protection that the donor cornea enjoys from the ocular immunological surveillance system of the recipient. Yet, it has been observed that some corneal transplants when placed in a nonvascularized host are rejected, which suggests that donor cells are capable of sensitizing the recipient and thus the concept of "privilege" is indeed a relative term.

Evidence has recently been collected showing that a distinct population of dendritic cells (Langerhans' cells) of mesenchymal origin resides on epidermal surfaces of many mammalian species and that these cells carry a histocompatibility antigen thought to be of central importance in the afferent arm of allograft rejection.[23] Gillette and associates[24] have determined that an equivalent subset of dendritic cells occurs in the ocular surface epithelium of several species through the use of enzyme histochemistry, immunochemical techniques, and electron microscopy. These investigators were also able to demonstrate the appearance of positive immunofluorescence on the surface of cells derived from human conjunctival and corneal epithelium when individual layers of these cells were reacted with an antibody prepared against human HLA-DR antigen.

To gain more insight into the role of the HLA-DR antigens in allograft rejection, Chandler and coworkers[25] took advantage of the extensive information available in the immunologic literature about analogous Ia antigens at the major histocompatability complex in the mouse (designated H-2) to develop a murine model for transplant studies. Through transplantation of whole corneas to subcutaneous pouches in the abdominal wall between various inbred strains of mice and evaluation of the clarity of such grafts, a two-signal model for corneal allograft rejection was proposed. This process can be described briefly as follows: Donor Ia antigen-bearing cells or other special antigen-bearing cells in the ocular surface epithelium cells provide not only a foreign antigen but also a second signal (possibly a soluble, hormonal factor) that are both required to activate cytotoxic T-lymphocytes which appear in the serum. These activated lymphocytes are

now capable of recognizing histocompatibility antigens on donor corneal cells and can lead to destruction of the allograft with accompanying rejection of the graft.

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CATARACT

INTRODUCTION

Cataract is a large public health problem in the United States and in the rest of the world. Because there are an increasing number of older persons living longer each year in this country, it is not surprising that senile cataracts are the third cause of existing and new cases of registered blindness in the United States.[1]

The NEI Cataract Program is divided into seven subprograms. The year's outstanding accomplishments in each of these subprograms are highlighted in this report. Particular attention is given to research areas that have been identified as Program Development Priorities in the National Advisory Eye Council's Vision Research--A National Plan: 1983-1987, of which Volume Two/Part Three is the "Report of the Cataract Panel."

PROGRAM STRUCTURE

Subprogram	FY 1984 Grants*
1. The Normal Lens	39
2. Epidemiology of Cataracts	1
3. Senile Cataract	32
4. Diabetic and Metabolic Cataract	14
5. Nongenetic Congenital and Genetic Cataracts and Dislocated Lenses	4
6. Cataract Induced by Environment and Toxic Effects	13
7. Treatment of Cataract and Correction for Aphakia	6
TOTAL	109

*Includes R01, R03, and R23

RECENT ACCOMPLISHMENTS

The Normal Lens

Molecular Biology. The field of molecular biology, especially gene structure and function, has made enormous progress through the development of recombinant DNA technology.

Cataracts of varied etiologies, including hereditary cataracts, are associated with altered levels of gamma-crystallins. In the past year, Bhat and Spector[2] succeeded in cloning different gamma-crystallin cDNA sequences and have determined the complete nucleotide sequence of a bovine gamma-crystallin mRNA. Gamma-crystallins are characteristically synthesized in the terminally differentiating lens fiber cells and, therefore, can be used as convenient markers of differential gene expression in the normal and cataractous states.

In evolution the alpha A_2 crystallin of the lens is highly conserved. McDevitt et al.[3] used previously cloned murine alpha A_2 crystallin cDNA (p M alpha Cr2) to isolate for the first time the human alpha A_2 crystallin gene. This permits sequence characterization and analysis of polymorphisms of this gene in individuals with normal lenses and with hereditary cataract.

Lens Transport Processes. There is a general need to understand the transport processes, both active and passive, involved in different lens membrane systems. Jernigan and Laranang[4] compared amino-labeled ^{15}N-glutamine with ^{15}N-glutamate as a source of nitrogen for amino acid metabolism in cultured rat lenses. The results indicated that the two-step process of glutamine transport and deamidation is more rapid than transport of glutamate. Lenses cultured with glutamine released sufficient ammonia into the culture medium to account for most of the added amido nitrogen.

Kern and Zolot[5] studied differences in the effects of group-reagents specific for sulfhydryl groups of lens proteins. The results indicate that cysteine residues of membrane-proteins are critically involved in mediated transport. They apparently have no role in transcellular diffusion of small polar molecules like water. There appears to be a basic difference between the systems in the lens that are responsible for passage of organic nutrients and active transport of Na^+ and K^+, compared with channels for inorganic ions.

Cell Division and Protein Synthesis. Research in this field is aimed at understanding the mechanisms that give rise to lens growth, cell differentiation.

As part of an investigation of the role of extracellular matrix on lens differentiation, McAvoy and Parmigiani[6] observed that laminin and fibronectin were localized during lens morphogenesis. Laminin and firbronectin are two important glycoprotein components of basement membranes and have been implicated in inductive interaction in embryos.

Lens fiber differentiation involves extensive cell elongation. Beebe and Snellings[7] noted that in chick embryos elongation is associated with a proportional increase in cell volume and is caused by lentropin, a protein found in vitreous humor of the eye. Lentropin caused a two-fold increase in ouabain-sensitive K^+ influx and a 50 percent decrease in intracellular Na^+ content.

Lens Proteins, Carbohydrates, and Lipids. Further studies of the composition, distribution, and metabolism of lens proteins, carbohydrates, and lipids are recommended in the National Plan, using both biochemical and noninvasive techniques when applicable.

Tao et al.[8] isolated five neutral glycosphingolipids from human cataracts using silicic acid column chromatography and preparative thin-layer chromatography. Three of these glycolipids were partially identified as glycosylceramide, lactosylceramide, and trihexosylceramide. This is the first report of neutral glycosphingolipids in human cataracts and the existence of a neutral fucolipid in the lenses of any species.

Garner et al.[9] measured the surface or solvent accessibility of certain individual aromatic residues of calf-gamma II crystallin by the dramatic intensity enhancements of NMR lines generated by the interactions of cyclic radical pair formation of the flavin I dye excited (488 nm) by an argon laser with the protein. This effect is called photochemically-induced dynamic nuclear polarization: photo-CIDNP. Their results suggest that while the four tryptophan residues are completely buried, His-113 and His-14 of the five histidines, and Tyr-165 and Tyr-62 of the fifteen tyrosines, are sufficiently exposed to elicit a photo-CIDNP effect.

Lens Morphology. Appreciable progress in understanding lens anatomy has been accomplished due to the use of transmission and scanning electron microscopy.

Macsai et al.[10] fractured, fixed, and dried adult frog lenses into two halves between adjacent radial cell columns (RCCs). They observed that the total amount of cell fusion as a function of radial location markedly increased with age. Membrane fluidity, gap junctions, presence of underlying cytoskeleton, and lack of basal lamina are all necessary for cell fusion to occur.

The existence of square arrays in lens fiber cell membrane is uncertain. The nature and functional role of the square arrays are completely unknown. Lo and Harding[11] described a systematic investigation of square arrays in various regions of the cortex and nucleus of intact human lenses using freeze fracture and thin section TEM. Structurally, square arrays were located only in non-junctional single membranes. They are structurally different from gap junctions found in the same region of the human lens. Their study strongly suggests that square arrays play a role in the development of the ridge pattern in human lens fiber cell membranes.

Lens and Aging. The National Plan states that further studies are needed of the physical, physiological, and biochemical changes in the lens as functions of age. Since the most prevalent human cataract is clearly related to aging, there is a great need to understand the aging process in the lens.

Lerman[12] stated that chronic cumulative exposure to 300-400nm UV radiation results in an age-related increase in a series of photochemically induced chromophores within the lens proteins and an associated increase in coloration of the lens nucleus. He demonstrated the feasibility of meas-

uring the age-related increase in lens fluorescence in vivo on more than three hundred normal individuals (1st to 9th decade) by UV slit lamp densitography.

Bursell[13] demonstrated that photon correlation spectroscopy is a sensitive method for nonivasive quantitation of protein changes in the in vivo human lens. The method has been applied to measuring lens protein alterations occurring during aging and as a result of diabetes. The results indicate that blood glucose levels should be considered in interpreting measurements made from the lens, especially if longitudinal monitoring of individual subjects is planned.

Gap Junctions, Cytoskeleton, and Cell Membranes. There is a need for studies of the composition and structure of the cytoskeleton and cell membrane, with particular attention to changes occurring during differentiation, cell elongation, and accommodation.[14]

Maisel and Ellis[15] identified actin, vimentin, and fodrin (spectrin-like proteins) in the human lens superficial cortical fiber cells of human lenses from birth to 90 years by molecular weight, isoelectric, and immunological properties. A rapid loss of vimentin was noted in deeper fiber cells at all ages.

Miller and Goodenough[16] examined the gap junctions from a large number of chick embryonic lenses. They observed that precisely at stage 24, the epithelial cells and lens fibers interconnect by gap junctions, and are demonstrably dye-coupled with Lucifer yellow. They pointed out that there appeared to be more than one class of gap junctions, and that the fiber/fiber junctions are truly unique, but they also demonstrated that an individual cell, the lens epithelial cell, can express two different junctional phenotypes, depending on its cellular neighbor.

Epidemiology of Cataract

One of the objectives in studying the epidemiology of cataracts is to obtain valid and reliable data on the incidence and prevalence of cataracts, visual impairments from cataracts, and their variations according to time, place, and subgroup of population.[17]

The hypothesis of an association between human cataracts and sunlight exposure, has been supported by recent studies.[18] Taylor et al.[19] are attempting to relate senile eye conditions to actual individual sunlight exposure, as determined by years worked, in two occupational groups with widely varying exposure conditions. They chose Maryland watermen and their wives and contrasted them to Pennsylvania coal miners and their wives. There appears to be an indication of an increased age-specific prevalence of lens opacities for watermen as compared to miners, with similar results seen in the wives.

Senile Cataract

Senile cataract is the opacification of the human lens that occurs with aging. There are consists of two main subgroups: cortical and nuclear opacities.

Environmental Factors. Garadi et al.[20] report an intriguing finding of the presence of crystallins linked by disulfide bonds to membrane proteins in the nuclei of X-rayed lenses prior to the formation of mature cataract. They hypothesize that the burst of oxidative damage that occurs in the mature X-ray cataract may begin at the site of nuclear membranes. The X-ray cataract may begin at the site of nuclear membranes. The X-ray cataract of the rabbit appears to be an excellent model for human senile cataract. The sequence of protein aggregation events appears to be similar in the animal and in the human.

Leveille et al.[21] record the effects of dietary restrictions on age-related distribution of gamma crystallin in the mouse lens. They followed the distribution of soluble gamma crystallins in mice with and without dietary restrictions from ages 2 to 30 months. The results suggest that dietary restrictions decelerated age-related loss of soluble gamma crystallins. Mice on restricted diets lived for some 19 months longer than those on the normal, unrestricted diet.

Culture System for the Human Lens. According to the National Plan,[22] the development of an in vitro culture system for the human lens and for lens epithelial cells is very important for seeking means to prevent, delay the progress of, or reverse the cataractous process.

Reddan et al.[23] are seeking to identify factors that regulate cell division, differentiation, and aging in mammalian lens epithelia. Endothelial growth factors have properties similar to those of eye derived growth factors. They found that endothelial cell growth supplement (ECGS) was a lens mitogen which evoked morphological changes in lens cells similar to those obtained by extracts of retina, iris, or ciliary body. When epithelial cells were cultured on fibronectin or laminin in serum-free minimum essential medium plus ECGS, insulin, and epidermal growth factor, a 33 to 40-fold increase in number was noted by day 13.

Development of Cataract Classification. A system for classifying the features of human cataract was proposed in 1978 and adopted by the U.S. Cooperative Cataract Research Group (CCRG) in 1980. White et al.[24] were concerned about the impact of cataract on the qualities of transmitted light. Thirty-five extracted human cataracts were photographed with black and white film according to the standard CCRG classification protocol and with a retroillumination device designed to approximate conditions used in in vivo retroillumination photography. A computerized image analysis was performed to produce histograms describing the distribution of gray levels comprising cataract images obtained with transmitted and reflected light. Their findings suggest that for some types of cataracts, retroillumination provides a significantly different type of photographic image, one which may provide more information about the lens and the functional impairment due to the cataract.

Biochemistry of the Lens. This broad area includes the study of the plasma membrane of normal and cataractous human lens fiber cells and its interaction with cytoskeletal and soluble lens proteins. In addition, it includes lens lipids, carbohydrates, and enzymes.

Taylor and Jahngen[25] reported that ubiquitin is a small polypeptide

(M_r 8500) which has been found in all cells in beef eye lens examined so far. Abnormal proteins appear to be degraded more rapidly than native protein and initiation of this proteolytic process is dependent upon the presence of ubiquitin and ATP. Since association and precipitation of oxidized proteins is related to cataractogenesis, it seemed plausable that altered proteolytic capabilities or cofactors might be related to opacification.

Ireland and Maisel[26] described how they incubated chick lenses in phosphate-free medium supplemented with ^{32}P-labeled orthophosphate and determined endogenous phosphorylation of lens proteins. They observed that the most acidic variants of vimentin were labeled in the epithelial and cortical fiber cells. When the water insoluble fraction of the whole lens was incubated with (gamma-^{32}P) ATP in the presence of cAMP, there was a marked enhancement of phosphorylation especially of the 47Kd beaded filament. These results indicate that many lens proteins are endogenously phosphorylated and that cAMP-dependent mechanisms are involved.

Light-Scattering Elements. Light scattering in senile cataract is probably caused by greatly increased levels of high molecular weight protein aggregates.

Clark and Danford[27] reported that elevated calcium levels decreased the transparency of cells in the lens cortex but not in the lens nucleus. They measured the relative transmittance by laser spectroscopy. They further noted that calcium has a stronger effect on the cortical cell membranes than on the cortical cytoplasm. Calcium did not decrease transparency in membrane or cytoplasmic fractions from the nucleus.

Ali and Bettelheim[28] tried to establish the forward scatter of light that reaches the retina and the back scatter of light that can be seen in slit lamp examination. Normal excised human lenses between the age of 3 and 85 were investigated. The forward scattering of excised human lenses showed little age dependence. The backward scattering of normal human lenses showed definite age dependence. They concluded that the intensities of back scatter in normal lenses do not necessarily reflect the light intensities reaching the retina.

Bettelheim and Christian[29] recorded that the cold cataract of calf lenses is a second-order phase transition. They calculated the energetics involved over a 1°C temperature change in the reversible cold cataract and they estimated similar energetics that would occur in irreversible cataract formation such as in syneresis. The irreversible cataract formation involves at least a magnitude larger energy change than the cold cataract formation. They concluded therefore that it is questionable whether cold cataract can serve as a good model for human cataractogenesis.

Antioxidant Defense Mechanisms. Direct evidence of the involvement of oxidation in cataractogenesis has been gained experimentally in animal models. One lens oxidant receiving considerable attention by researchers is hydrogen peroxide.

McGready and Giblin[30] reported that rabbit lens can tolerate exposure to a physiological concentration of hydrogen peroxide without the

accumulation of oxidized glutathione (GSSG). When the lens is pretreated with 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU), a compound which inhibits glutathione reductase and blocks hydrogen peroxide-induced stimulation of hexose monophosphate shunt activity, exposure to the same concentration of hydrogen peroxide produced significantly increased levels of GSSG. The activity of lens Na-K ATPase was unaffected by exposure to either hydrogen peroxide or BCNU alone. However, when lenses were first treated with BCNU and then exposed to hydrogen peroxide, there was nearly a 40 percent decreased Na-K ATPase activity. Their results indicate that lens Na-K ATPase can be inactivated by a physiological concentration of hydrogen peroxide when the metabolism of glutathione is inhibited.

Barnett et al.[31] noted that both glutathione reductase and aldose reductase (AR) use NADPH as a cofactor. They studied the interaction of these two enzymes. They incubated rat lenses in various concentrations of glucose either with or without hydrogen peroxide. They measured ^{13}C NMR spectra during the incubation. They found that with oxidative stress the V_{max} of aldose reductase was lower. Their conclusion was that there was a possible role for oxidation in diabetic cataract formation.

Diabetic and Metabolic Cataract

Research in this subprogram is concerned with studies of cataracts produced as a result of diabetes or the presence of excess galactose or other types of sugars in the blood.

Metabolic cataracts are those which may result from a specific metabolic block or abnormality; in the broadest sense they include sugar, nutritional, and genetic cataracts.

Drugs to Delay Diabetic Cataract. Although enhanced activity of the polyol pathway has been implicated in certain complications of diabetes, such as in cataract, evidence that aldose reductase activity and sorbitol content are increased in diabetic renal lesions has been lacking. Beyer-Mears et al.[32] measured polyols in glomeruli isolated from control and streptozotocin-diabetic rats, and assessed whether changes in diabetic glomeruli could be prevented by oral administration of the aldose reductase inhibitor sorbinil. They found that glomeruli contain aldose reductase activity and provided the first demonstration that glomeruli polyol content increases while myo-inositol content decreases in diabetes. They also discussed that oral sorbinil prevents these changes despite persistent hyperglycemia.

Pathways in Diabetic and Metabolic Cataracts. An understanding of the nature of diabetic cataracts is evolving from detailed studies of sugar cataracts either produced experimentally or found in some animal models with congenital diabetes.

Normal glucose metabolism is fundamental to the maintenance of lens transparency. Wolfe and Chylack[33] examined several factors affecting glucose metabolism with the goal of developing quantitative relationships describing the rate of glucose utilization and its distribution among its metabolic possibilities. Increasing the ATP concentration from 1 to 3 mM resulted in an 80 percent decrease in lactate production and an increase in

the glucose-6-phosphate level. The addition of NADH to the incubation resulted in increased alpha-glycerol phosphate production. These data were consistent with feedback control of glycolysis by ATP. Furthermore, the reduced coenzyme is capable of directing various pathways of glucose metabolism.

Studies have suggested that heterozygotes for galactosemia show relatively low levels of galactokinase (GK) and/or galactose-1-phosphate uridyl transferase (GPUT) activity. This may predispose them to the development of metabolic presenile cataracts via the aldose reductase pathway. Stevens et al.[34] determined the erythrocyte activity of these enzymes in cataract patients and controls. The patients' conditions were severe enough to warrant surgery on or before age 55. The results showed no statistically significant difference between cataract and control GK or GPUT activity. However, there was a trend for lower GPUT activity in the cataract group.

Role of Nutrition in Cataract. Hess et al.[35] reported that nuclear opacity appeared in rat pups 72 hours following a single injection of Na_2SeO_3 and is preceded by a three-fold accumulation of Ca^{++}. Their studies indicated that divalent ion-dependent proteolysis occurs in these young rat lenses and that the appearance of low molecular weight peptides in the selenite-induced cataract may result from the elevated Ca^{++}.

Protein Metabolism in Cataracts. Belisle et al.[36] prepared monoclonal antibodies to rat lens-soluble protein $beta_H$ (FII). Spleen cells from BALB/c mice injected twice with FII were fused with non-secreting clones of mouse myeloma cells. Five of the monoclonal antibodies, so produced, demonstrated in the ELISA assay no cross reactivity with the other crystallin fractions.

Nongenetic Congenital and Genetic Cataracts and Dislocated Lenses

Congenital cataracts fall into two broad categories: those which are nongenetic but acquired during uterine life, and genetic. Nongenetic cataracts are usually accompanied by other ocular abnormalities, such as pepper and salt retinopathy, or by extraocular anomalies, such as deafness or cardiac disease, or even mental retardation which is evidence of widespread intrauterine viral disease. Genetic cataracts can be present at birth or develop at a later developmental stage as infantile or juvenile cataracts.

Genetic Cataracts in Families. Cats which were monocularly deprived during a critical period in development have poor acuity in the deprived eye and little binocular vision. The longer the deprivation, the poorer the outcome.

Brent et al.[37] found a similar pattern of results with children who had been deprived of normal visual experience because of dense cataract in one eye either present at birth or induced by an injury after the age of three. The cataracts had been removed surgically and the aphakic eyes fitted with contact lenses which corrected the refractive error. Within the congenital group, children who had had the earliest treatment followed by patching of the normal eye had the best outcome. In contrast, within

the traumatic group, most children had good Snellen acuity in the treated eye and some binocular function even when the deprivation was of long duration and there was little patching of the normal eye.

Cultured Lens Epithelial and Fiber Cells. Muggelton-Harris[38] obtained exciting results in in vivo mouse embryo studies from a number of chimeras formed from cataractous and normal mouse embryos. It appears that the cataractous state can be modified, and the allophenic mouse does not acquire the genetically dominant Fraser cataract or the developmental Nakano cataract defect at the time when these abnormalities normally appear, during differentiation and development of the lens cells and embryos.

Another experiment made in vivo studies of aging and cataractogenesis in normal mice (C57BL/6). She provided evidence that lysosomal enzyme N-acetyl-glucosaminidase activity in the lens epithelial cells does decrease with age. Such changes in lysosomal enzymes could be important to cataractous formation.

Molecular Genetic Probes. Kang et al.[39] purified from calf lens a novel nucleic acid binding factor, designated as RF-32. Polyclonal antibodies were obtained from rabbits immunized against purified RF-32, and the serum IgG fraction was prepared. Monoclonal antibodies were also prepared by standard hybridoma technique. They observed that antibodies to RF-32 can recognize both double-stranded (ds) and single-stranded (ss) nucleic acids, although the binding to ds nucelic acids is stronger. In addition, anti-RF yields a positive reaction with Z-DNA, a left-handed Z-helix DNA. These results indicate that RF-32 contains specific sequence determinant(s) which can induce the anti RF-32 to recognize specific nucleic acid conformation.

Zonular Fiber. The ocular zonules are still incompletely characterized biochemically. Streeten and Licari[40] proposed that elastic fibers occur in some tissues as a three-part interconnecting system. The system includes two sizes of elastin-containing fibers surrounded by tubular microfibrils (elastic microfibrils), besides isolated bundles of tubular microfibril without elastin (oxytalan fibers). This little-studied system was identified in the bovine ciliary body by light and electron microscopy. Its architecture varied regionally, suggesting different vectors of tractional force in the anterior and posterior ciliary body related to accommodation. Zonular fibers had the staining characteristics of oxytalan fibers, and their fibrils were ultrastructurally similar to the tubular microfibrils around elastic fibers and those composing oxytalan fibers. Antibodies to microfibrillar protein bound to zonules and to tubular microfibrils in all sites. This was the first evidence that tubular microfibrils, both with and without elastin, share antigenic determinants; this confirms the close antigenic relationship of the zonules to this class of proteins.

Cataract Induced by Environmental and Toxic Effects

The lens, which continues to grow throughout life, does not renew its cells; mature fibers are expected to remain transparent through a person's lifetime. Continuous insult to the lens from drugs or radiation may interfere with the normal repair and protective mechanism, thereby hastening the formation of senile cataract.

Cataractogenic Effect of Radiation. Studies of the damage to the lens and retina caused by light have been accelerated recently. Zigman[41] stated that the evidence that near-UV radiation stimulates cataract formation is very strong. Avoidance of excessive exposure to near-UV light and the use of protective lenses that filter it out are suggested to prevent the enhancement of human cataract formation by near-UV light.

Borkman et al.[42] exposed whole rat lenses in vitro to unfocused, monochromatic 337.1 nm laser radiation, and the resulting changes were measured using UV absorption, fluorescence, and SDS-PAGE analysis. The experiments demonstrated that it is possible to deliver sufficient UV radiation to an intact lens in the laboratory, with no added photosensitizer, to cause measurable damage. Some of the photochemical changes observed resemble changes accompanying human aging and cataracts. In further experiments they photolyzed at 337.1 nm lens crystallins and the free amino acid tryptophan. Irradiation of lens crystallins at 337.1 nm resulted in increased UV absorption at 250 nm and 320 nm, decreased fluorescence at 335 nm, and alterations in SDS-PAGE pattern. All of these changes were similar to those observed following lens protein irradiation at 290 nm. This similiarity had not been expected, since 290 nm radiation is absorbed primarily by tryptophan residues in lens proteins, whereas 337.1 nm radiation is expected to be absorbed not by tryptophan but by unknown photosensitizers in lens and lens proteins. The investigators subsequently found that the free amino acid tryptophan could also be photolyzed using 337.1 nm radiation.

Systems for Study of Radiation and Toxicity. Bhuyan and Bhuyan[43] found that Adriamycin (ADM), an anthracycline quinone, was a potent cataractogenic agent in rat neonates. The hydrogen peroxide of aqueous humor was significantly higher. Malondialdehyde (MDA) increased in early cataract and in advanced cataract as compared to controls. In addition, there was oxidation of lens thiols. Exposure to ADM indicated enhanced production of singlet oxygen by generation of ADM semiquinone free radicals. Formation of oxygen free radicals and hydrogen peroxide by ADM, causing lipid peroxidative damage to the lens, was also seen in vitro.

Jernigan and Laranang[44] observed that photosensitized oxidation of cultured rat lenses greatly decreased their ability to accumulate labeled choline from the medium. This effect required a photosensitizer (riboflavin), light, and oxygen. Choline accumulation appeared to be somewhat more sensitive to damage than ^{86}Rb accumulation. The photooxidation also caused a progressive and concurrent decrease in both ATP concentration and choline phosphorylation. It appeared that the early decrease in accumulation of ^{3}H-choline in these lenses resulted from effects on transport rather than from decreased conversion to phsophorylcholine.

Mode of Action of Cataractogenic Drugs. Kuszak et al.[45] induced experimental cataract in rat lenses by subcutaneous injection of U18666A, a potent inhibitor of cholesterol biosynthesis. They made extensive studies of control and experimental lenses by stereo transmission electron micrographs. Their results suggested an alteration of lens membrane in general and specifically a breakdown of gap junctions during the cataractogenesis. They did not observe a single gap junction in the advanced cataractous lenses. These was findings were consistent with SDS-PAGE studies of membrane from lenses with cataract induced by U18666A, which shows a loss of a main intrinsic protein (MIP) 26K, the principal component of lens gap junction, and the appearance of a 22K membrane protein that is thought to be a breakdown product of MIP 26K.

Treatment of Cataract and Correction of Aphakia

At present there is no medical treatment proved capable of preventing or curing cataract. Thus, surgery to remove the cloudy lens is the only available alternative.

Cataract Surgery and Aphakic Correction. In the last few years in the United States there has been a definite trend toward greater use of intraocular lens implants (IOLs) after cataract surgery. The long-term effects of the interactions between the intraocular lens and the immune system of the implanted patient are not yet clear. Tuberville et al.[46] presented the results of aqueous humor cytological analysis of patients undergoing intraocular surgery for cataract, and of patients who were aphakic (ABK) or had pseudophakic bullous keratopathy (PBK). The PBK patients were divided into two categories: those with anterior chamber pupillary lenses, and those with posterior chamber lenses. The aqueous humor from the cataract patients was acellular. In that from ABK patients, cell counts were much smaller than in aqueous humor from PBK patients with anterior chamber or pupillary lenses. PBK cell counts with posterior chamber lenses were similar to ABK cell counts. More than 99 percent of the nucleated cells were of the activated monocyte-histiocyte type. One PBK patient with a pupillary lens had many multinucleated giant cells in the AH.

Lens Accommodation. Accommodation is the neurophysiologic process by which the eye automatically refocuses when visual attention changes from distant to near objects. A continuous decrease in accommodative amplitude and the development of presbyopia are generally considered to be inevitable consequences of aging. Koretz et al.[47] investigated the changes in curvature of the human crystalline lens as functions of accommodative state and increasing age using slit-lamp photographs taken from human subjects aged 11 to 45. All curves, external boundaries, and bounds between adjacent zones of discontinuity inside could be fitted to a simple equation. The sharpness of change in curvature increases for a given lens with increasing distance from the lens surface, with increasing accommodation, and with increasing age, while the size of the region within each lens that has approximately spherical curvature decreases. In contrast, the anterior of the presbyopic lens (age 45) appears to be "frozen." This is the first direct evidence of alteration in internal lens function with presbyopia.

In a different approach on the same subject, Kaufman et al.[48] used in vivo slit lamp photographs of nonaccommodated rhesus lenses undergoing carbachol-induced accommodation using a tilted film plane camera. The animals represented the entire age range for this species. Some were surgically aniridic, permitting detailed study of internal lens structure at various ages and accommodative stages. Uncorrected external and internal lens curvature were digitized and fit to equations. Rhesus internal lens structure differs from the human by exhibiting only two high density regions, one each posteriorly and anteriorly. However, as in the human, all observed curvatures can be fit to an equation of the form $y = a + cx^2$. Sharpness of anterior surface curvature progressively increases up to approximately 18 to 20 diopters of accommodation, then remains essentially unchanged with further accommodation. Sharpness of posterior lens curvature and lens thickness also increase up to approximately 18 to 20 diopters of accommodation, then appear to decrease with further accommodation.

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GLAUCOMA

INTRODUCTION

The research priorities of the glaucoma program, as set forth in Vision Research--A National Plan: 1983-1987, emphasize both clinical and basic approaches to better understanding and improved management of the various types of glaucoma. Major progress reported during the past year has been in three subprograms. Primary open-angle (including clinical studies), aqueous humor inflow and outflow, and means for determining early damage to the optic nerve. Less research activity occurred in the other subprograms, due in part to the clinical success of laser iridotomy (angle closure glaucoma), to lack of promising research opportunities (congenital glaucomas), and lack of a suitable model system (neovascular glaucoma). Efforts have been made to stimulate interest in research on the molecular biology and genetically specific cell membrane components of the trabecular meshwork endothelial cells and ciliary epithelial cells. The Glaucoma Laser Trial, a controlled multicenter clinical trial of argon laser trabeculoplasty in newly diagnosed glaucoma, is under way.

PROGRAM STRUCTURE

Research on glaucoma is classified by subcategories of the disease. Because primary open-angle glaucoma is the predominant type of glaucoma, elements common to all types of the disease are considered under this heading.

Subprogram/Area	FY 1984 Grants*
1. Primary Open-Angle Glaucoma	
a. Etiology, Epidemiology, Management, and Therapy	14
b. Aqueous Humor Dynamics: Inflow	36
c. Aqueous Humor Dynamics: Outflow	19
d. The Optic Nerve	16
2. Other Glaucomas	
a. Angle Closure Glaucoma	3
b. Developmental, Congenital, and Infantile Glaucoma	1
c. Secondary Glaucomas	12
TOTAL	101

*Includes R01, R03, R13, and R23, K11 mechanisms.

RECENT ACCOMPLISHMENTS

Primary Open-Angle Glaucoma

Etiology, Epidemiology, Management, and Therapy

Compliance with drug therapy. The extent of patient compliance with prescribed drug regimens and drug administration is extremely difficult to evaluate, yet the success of therapy may be critically dependent upon it. Kass has undertaken an ingenious study, combining observations of how patients apply topical ocular drugs after instruction, use of questionnaires, and tracking of drug use with a computerized drug applications monitor.[1] Patterns of pilocarpine use are being followed in patients instructed to use drops 4 times daily. Nearly all of 108 patients reported taking the drug over 97 percent of the time as instructed, and only 2 percent reported using less than 75 percent of doses. The monitors showed that on the average, only 76 percent of doses were used, 6 percent of patients took less than 25 percent of their doses, 20 percent less than half, 37 percent less than three-quarters, while 2 percent of patients overdosed themselves. Spacing of drug applications was also very divergent: less than half the patients followed the prescribed dosing intervals. Additionally, the physician's predictions of an individual's degree of compliance correlated very poorly with the measured rates. Thus, the vital therapeutic relationship between the physician's instruction and expectation of drug use and the individual patient's response is very weak and is deserving of considerably more attention.

Zimmerman et al. instructed patients in the techniques of drop application, nasolacrimal closure, and eyelid closure. They monitored the effects of timolol in a masked, random crossover study.[2] Use of these techniques significantly reduced systemic absorption of timolol (by 60 percent), indicating that systemic side-effects can be reduced by proper application of drugs, and clinical efficiency was demonstrated by diminished aqueous flow as measured by aqueous humor fluorometry.

Development of a home-use tonometer. Knowledge of diurnal variations in a patient's intraocular pressure would greatly aid in diagnosis of the disease and in prescription of maximally effective therapy. Most measurements are made during a physician's office hours, often at random times, while diurnal studies have been conducted on hospitalized patients. Zeimer devised and tested a tonometer designed for a patient's use at home. Representative cases showed expected diurnal curves, differences between the two eyes, variations in time of maximal and minimal intraocular pressures, and erratic daily swings in pressure.[3] It remains to be seen if prescribing application of drops to coincide with pressure maxima will improve glaucoma treatment.

The Glaucoma Laser Clinical Trial. This is a multicenter study, to evaluate the efficacy and safety of argon laser trabeculoplasty in comparison with the use of topical medications. In the first year, all study centers and personnel were certified to perform the study protocols; the procedures were thoroughly analyzed, tested and "debugged"; and recruitment is now under way. It now appears, that the projected rate of recruitment of patients with newly diagnosed glaucoma was low. As a consequence of

this, an announcement that new clinical centers will be added has been circulated. This study will require major efforts at recruitment and cooperative referrals from ophthalmologists and optometrists in the communities in which it is based.[4]

Improvement of filtering surgery. Scarring over a filtering bleb is a common cause of failure of glaucoma surgery. Histopathologic studies of eyes after failed filtering operations have suggested that proliferation of fibroblasts and deposition of collagen constitute a barrier to filtration. Gressel et al. reasoned, therefore, that inhibition of fibroblast proliferation might improve the success rate for this procedure.[5] They tested the effects of subconjunctival injections of 5-fluorouridine, an anti-metabolite, on experimental filtering blebs in monkeys with promising results. Very encouraging results were also obtained by Heuer et al. in a pilot study on humans who had poor prognoses for filtering surgery--in aphakia, neovascular glaucoma, and in phakic eyes following two unsuccessful filtering operations.[6]

Marihuana Update. Interest continues in the therapeutic potential of marihuana components, such as delta-9-tetrahydrocannabinol (THC), that may be free of the central nervous system (CNS) and behavioral effects of cannabinoids. A major objective of the following studies is to determine if the intraocular pressure-lowering effects of plant extracts and various semi-synthetic compounds can be dissociated from their undesirable side-effects.

Waller[7] is continuing to examine natural cannabinoids and some derivatives, testing them first in rabbits, then in monkeys. Two such compounds, cannabidiol and canniprene, lowered IOP modestly and showed no CNS activity in screening tests. Of some interest is this group's earlier finding of anti-inflammatory activity of two cannabinoids in standard screening tests. Now these investigators have extended these observations to the eye, studying arachidonic acid-induced ocular inflammation and the accompanying rise in pressure. Cannabinene and cannabidiol afforded some protection, while THC had no effect. The clinical usefulness of these observations remains to be determined.

Colasanti has been studying the pharmacology and toxicology of cannabinoids in cats.[8] Cannabidiol chronically administered by a minipump lowered IOP and produced no ocular or neurotoxic side-effects; however, tolerance to the pressurelowering developed within 6 days. Neurotoxic effects were dissociated from IOP reduction in tests with 11-hydroxy-THC. In these studies, no evidence for cholinergic or adrenergic modulation of THC effects was seen.

The ocular pharmacology of non-cannabinoid aqueous extracts of marihuana leaf and other plants isolated by Zalkow and Deutsch[9] is being examined by Green,[10] and their collaborative results are being reported jointly. These water-soluble compounds, which exert profound pressure-lowering effects, appear to be aggregates of high molecular weight glycoproteins. They are being further purified and characterized, and the properties responsible for their ocular hypotensive activity are being defined. In rabbits, aqueous humor inflow is reduced without any corresponding changes in blood pressure, PO_2, or body temperature. At low dos-

ages, an initial hypertensive effect is followed by a prolonged reduction in IOP. An interesting, and possibly significant, finding is that extracts of a number of other plants, including burley tobacco and kale, have ocular hypotensive effects of the same magnitude as the marihuana leaf extracts.

Aqueous Humor Dynamics: Inflow

New drug developments. There has been renewed interest in development of topically effective carbonic anhydrase inhibitors (CAIs), and a number of investigators are reporting varying degrees of success. Presently available CAIs are administered orally and cause a wide spectrum of side effects which limit their use. Maren and his colleagues are defining the biochemical and biophysical properties for effective corneal penetration and enzyme inhibition for this class of compounds.[11] Schoenwald is examining the therapeutic potential of ethoxazolamide analogs, and has applied for patents on two of these substances. The 6-hydroxyethyl derivative penetrated the cornea well, showed minimal toxicity, and had good ocular hypotensive activity.[12] Another series of compounds, differing in structure from conventional sulfonamide CAIs by being N-substituted, was more active than acetazolamide, possibly via a pro-drug mechanism.[13] The properties of a commercially developed topically effective CAI were described, at the 1984 meeting of the Association for Research in Vision and Ophthalmology, as having potent ocular hypotensive activity, no contralateral effect, and being solely attributable to reduction of aqueous humor flow.[14] Interest is continuing in finding ways to apply topical acetazolamide effectively. Friedman and Allen[15] used extended-wear contact lenses soaked in acetazolamide and reported a significant fall in IOP lasting to four hours without any contralateral effect.

Biophysical properties of ciliary epithelium. This has been an underrepresented area of glaucoma research, as noted in Vision Research--A National Plan: 1983-1987. Only recently have modern biophysical techniques been applied toward furthering our understanding of the physiology of the ciliary epithelium.

Green has initiated a series of studies of the electrical properties of ciliary tissues.[16] In vitro single cell recordings showed that both pigmented and non-pigmented cells have potentials of -65 mV and that all ciliary epithelial cells are electrically and anatomically coupled. Also, the non-pigmented (and possibly pigmented) cells have Na^+-K^+ pumps in their basolateral membranes. These data differ considerably from those reported 20 years ago. Hydraulic conductivity studies showed that normal cell permeability depends upon either intact metabolic pools or ionic permeability. Bathing solutions lacking Na^+, K^+, or HCO_3^- increased conductivity. In vivo treatment with adrenergic agonists caused loss of in vitro tissue responses after three days, indicating that B-agonists desensitize some process coupled to hydraulic conductivity.

Pessin and Candia also presented evidence for Na^+-K^+ pumps in the basolateral membranes of both types of ciliary epithelial cells.[17] Ion fluxes were measured in isolated rabbit iris-ciliary body under short-circuit conditions, and no net Na^+ or Cl^- flux could be identified.

Krupin et al. measured the transmural electrical properties of rabbit iris-ciliary body preparations, and found that the aqueous side was negative to the blood side by -1.2 mV.[18] Maintenance of the potential difference required Na^+, K^+, and HCO_3^-, and in the absence of Na^+ and K^+ the respiratory rate of the tissue diminished. Both Na^+,K^+-ATP-ase and bicarbonate ion were required for active ion transport in this preparation.

Aqueous Humor Dynamics: Outflow

Interest continues to be focused on the aqueous humor outflow pathways as the major determinant of elevated intraocular pressure in glaucoma. Considerable effort is being devoted to studies of the biology of the outflow pathways and to the properties of cultured trabecular meshwork endothelial cells. New information and some new investigative approaches are discussed in the following sections.

Effects of glycoproteins on aqueous humor filtration. Two sets of investigators presented evidence that may be interrelated and bear upon mechanisms raising IOP. An effect of aqueous humor components on outflow is suggested by use of a model system in Kamm's laboratory. Although the viscosity of aqueous humor is close to that of water, it flows through microporous filters at rates 2-10 times lower, and the rate of flow is inversely proportional to the volume of aqueous used. Hyaluronic acid does not seem to be involved in this phenomenon as hyaluronidase has no effect upon it; however, treatment with the proteolytic enzyme papain increases the rate of aqueous flow through the pores, suggesting that glycoproteins may in some way regulate the outflow process.[19] This same laboratory calculated that, based upon reported glycosaminoglycan (GAG) concentrations, and theoretical considerations, resistance to outflow cannot be fully accounted for by GAGs and that proteins too are involved.[20] Additional evidence for the role of proteins in regulating outflow resistance comes from Moses.[21] Eyes perfused in vitro with an inhibitor of fibrinolytic activity showed a significantly lower increase in outflow "washout effect" than control eyes. Worthen, Cleveland, and Slight showed that plasma fibrinectin from glaucoma patients specifically binds to type II collagen at significantly lower levels than normal plasma. This observation suggests that this or some other glycoprotein may bind differentially to the collagen beams of the trabecular meshwork to affect the rate of filtration of aqueous humor in normal and glaucomatous eyes.[22]

Effects of glaucoma medications on cultured trabecular cells. Tripathi and Tripathi applied "pharmacologic" concentrations of timolol, epinephrine, and pilocarpine to primary cultures of trabecular epithelial cells. Timolol enhanced the proliferation and growth of the cells without appreciably affecting their morphology or contractile properties, and pilocarpine produced no significant effects. However, epinephrine caused a cessation of cellular activity and rounding of the cells with breakdown of stress fibers, and by 48 hours significant degenerative changes were observed in the cells.[23] These findings provide a morphologic rationale for the clinical observation of subsensitization to epinephrine and complement pharmacologic studies indicating drug-induced loss of epinephrine receptors.

Neuroregulation. Little evidence for neuroregulation of trabecular meshwork function exists, yet neurotransmitters are present in aqueous humor and they do affect outflow. Stone and associates[24] have used immunohistochemical techniques to identify a marker, specific for neuroregulatory (probably neurosecretory) cells, on trabecular meshwork specimens. Positive reactions were observed in the anterior trabecular meshwork in cells which correspond to the Schwalbe's line cells described by G. Raviola.

Drug effects. As noted in last year's Annual Report, Bito and associates found that at low dosages topically applied prostaglandins (PGs) significantly lowered IOP in experimental animals. They have sought a patent and are exploring the therapeutic applications of these findings. Lee and colleagues have confirmed these observations and investigated the mechanisms involved. They found that in treated cat and monkey eyes outflow facility was significantly increased over control eyes. Fluorometric measurements also indicated increased uveoscleral outflow and no change in rate of aqueous humor inflow. In cat eyes, increased aqueous humor protein levels indicated some breakdown of the blood aqueous barrier.[25]

The Optic Nerve

High among the priorities recommended for glaucoma research in Vision Research--A National Plan: 1983-1987 are the development of new noninvasive methods for measuring optic nerve status, including psychophysical tests, and the means for early detection of glaucomatous changes in ocular hypertension.

Noninvasive methods and tests. Trick has been investigating the applicability of pattern-reversal retinal potentials (PRRP) to detection of early glaucomatous changes in ocular hypertension.[26] Optimal stimulus parameters have been defined and test results to date show that the amplitude of PRRP is significantly reduced in glaucoma patients compared with age-matched control subjects. Age was found to be a significant determinant of PRRP, with individuals in the 70-80 year old group showing values as low as half of those seen in 20-30 year olds. Now these measurements are being assessed in ocular hypertensives.

Studies of flicker threshold, yielding values for dynamic contrast sensitivity (DRC) and pattern visual evoked potential latency (PVEP), are being conducted by Atkin et al. in the same three classes of people.[27] These investigators have found that mean PVEP latency and DRC values differed significantly among normal, ocular hypertensive, and glaucomatous groups. These tests measure responses of the central degrees of the visual field, and it is of interest that in the glaucomatous, but not the hypertensive, eyes there was a positive correlation with cup/disk ratios, suggesting that the two populations have different characteristics.

Phelps and Motolko, also using contrast sensitivity measurements, found that central visual function is impaired in glaucoma.[28] The fact that contrast sensitivity is impaired, even when visual acuity is normal and visual field defects are far from fixation, may make this test a useful

clinical tool; however, there is overlap in values between elderly normal persons and glaucoma patients, again showing the importance of age in these types of measurements.

Because automated perimeters are being rapidly accepted into clinical practice, it is important to interpret the data they provide. Although automated perimeters offer many advantages over manual perimeters and can provide valuable clinical information, interpretation of the test results requires analytical clinical skills. Understanding the limitations of the machine printouts is emphasized by Wilensky and Joondeph, who studied the reproducibility of the visual field measurements obtained with the Octopus perimeter from a group of normal subjects.[29] Four sets of readings were made for each eye within a four week period. In 11 of 12 eyes, results for at least one point (range 1-6) varied more than 4 dB (range 5-11dB) among the four readings, and 7 eyes had points at which the light threshold was at least 5 dB lower than age-adjusted normals. This calls into question the interpretation of the "normal" and "abnormal" fields defined for this perimeter, since it appears that a 2 dB loss cannot arbitrarily be defined as signifying visual field loss.

Mechanism of nerve damage. Lively differences of opinion exist regarding the mechanism of nerve damage in response to elevated IOP, whether it be mechanical or a consequence of ischemia. In Barany's laboratory, the effects of increased IOP on axonal transport in the rat eye, which has a single lamellar sheet, was studied.[30] Horseradish peroxidase injected into the lateral geniculate nucleus undergoes retrograde axonal transport to the opposite retina. Following HRP injections, pressure in the eye was kept at 20, 35, or 50 mm Hg., and the distribution of HRP was measured. The amount of HRP in these tissues was diminished by 29 percent and 76 percent, respectively, compared to those of the eye kept at 20 mm Hg. It appeared that increased IOP itself can inhibit fast retrograde axonal transport in a way independent of circulation (ischemic damage) or lamellar shearing (mechanical damage).

Next, a very high IOP, 180 mm Hg., was maintained in rat eyes for a short period, 10 minutes, to test in vivo whether pressure reversibly disrupted axonal transport elements. HRP was injected into the lateral geniculate nucleus, and 3 hours later the IOP was raised. At various times the HRP in the retina was measured. This design excluded accumulation of HRP at the lamina cribrosa during the period of high pressure. It was expected that very high IOP would act to force HRP out of the eye by locally stretching the axons and rupturing cytoskeletal elements. However, there was complete recovery of transport 2 hours after pressure release. It was concluded that if the transport system became less efficient at high IOP, nutrient requirements of the nerve might not be met, suggesting that a combination of mechanical factors and impaired nutrition acts to damage the eye at elevated pressures.[31]

Bunt-Milam investigated the contribution of mechanical factors to optic nerve damage using the buphthalmic rabbit, which suffers an inheritable form of glaucoma. Since the rabbit has no (or only a poorly formed) lamina cribrosa, this model would serve to differentiate the effects of mechanical damage from an ischemic mechanism in determining

how elevated pressure diminishes axonal flow.[32] Recessive bubu rabbits and control littermates were used. The eyes of growing animals were obtained at different ages and examined by light and electron microscopy. Results indicated that in the absence of a lamina cribrosa no significant impairment of axonal transport was seen even though IOP was elevated, lending support to the mechanical theory of optic nervehead damage resulting from elevated intraocular pressure.

Other Glaucomas

Angle-closure glaucoma. Little new research was reported in this area in the past year. The success of argon laser iridotomy seems to have diminished research efforts in all areas save the search for a more efficient tool, where work was focused mostly on the NdYAG laser. However, no truly definitive study has emerged in this regard.

Developmental, congenital, and infantile glaucomas. This is an area of great clinical interest, but because of a lack of promising new experimental approaches, there is little true research activity apparent.

Secondary glaucomas. Some interesting approaches to understanding the mechanisms of glaucoma secondary to anterior uveitis appeared in the past year. Shapiro and associates used an endotoxin-induced model for uveitis in the rabbit eye, and a specially designed fluorometer was employed to assess the integrity of the blood-aqueous barrier.[33] A highly significant difference was measured between the experimental eyes and the controls, with the larger amount of fluorescein entering the experimental eyes. These changes were measured before flare could be seen by slitlamp in the treated eyes. In a similar model, Wong et al. attempted to identify the chemotactic contribution to cellular exudates in the anterior chamber. They reported that chemotaxis was the primary mechanism responsible for migration of polymorphonuclear cells into the chamber in this type of inflammation.[34]

O'Rourke's group is continuing to investigate the role of fibrin metabolism in mycobacterium-induced anterior uveitis. Earlier work has shown that fibrinolysis was diminished in the cat eye in this condition. In neither experimental nor control eyes was plasmin activity noted; however, in experimentally inflamed eyes there was significantly more plasminogen. Addition of exogenous plasmin or plasminogen potentiated fibrinoysis in both sets of eyes, suggesting the existence of a novel plasminogen activator in cat aqueous humor.[35]

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STRABISMUS, AMBLYOPIA, AND VISUAL PROCESSING

INTRODUCTION

Seeing involves a series of highly complex processes. These processes include searching and scanning eye movements to localize the image onto the retina, vergence and accommodation to refine the localization and to achieve focus, and visual processing, so that objects are actually perceived in detail, depth, and color. A disturbance of any of the many parts of these elaborate and precise systems can lead to serious visual disturbances, such as amblyopia, visual field defects, strabismus, nystagmus, and myopia. Although disorders of these systems may not always cause total blindness, they nonetheless may seriously diminish the professional opportunities and the quality of life of those they afflict. These conditions affect more than 10 percent of the population and thus constitute serious public health problems.

The National Eye Institute's Strabismus, Amblyopia, and Visual Processing program supports research on the structure, function, and development of the extraocular muscles and those portions of the brain that make vision possible. This research is directed toward gaining a better understanding of normal vision and of the causes of visual deficits and blindness that do not appear to result from specific dysfunction of the eye itself.

During FY 1984, the Strabismus, Amblyopia, and Visual Processing Program awarded grants to individual investigators with support of research in the following areas. The distribution of grants by subprogram areas is presented in the following table. Highlights of research accomplishments during the past year for several of these areas are presented below.

PROGRAM STRUCTURE

Subprogram/Area	FY 1984 Grants*
1. VISUAL PROCESSING AND AMBLYOPIA	
a. Normal and Abnormal Development	
(1) Molecular	16
(2) Cell and Systems	51
(3) Behavior	8
b. Structure and Function	
(1) Molecular	10
(2) Cell and Systems	63
(3) Behavior	29
c. Disorders	
(1) Amblyopia	17
(2) Sensory Neuro-Ophthalmic Disorders	0

2. OCULAR MOTILITY AND STRABISMUS	
a. Normal and Abnormal Development	6
b. Structure and Function	
(1) Conjugate Eye Movements	32
(2) Vergence and Accommodations	6
(3) Muscle Structure and Physiology	6
c. Disorders	
(1) Strabismus	24
(2) Motor Neuro-Ophthalmic Disorders	8
3. OPTICS AND REFRACTIVE ERRORS, INCLUDING MYOPIA	
a. Optics and Refractive errors, including myopia	5
4. VISUAL IMPAIRMENT AND ITS REHABILITATION	
a. Visual Impairment	4
b. Rehabiliation	1
TOTAL	286

* Includes R01, R03, R13, R23, K04, and K11 mechanisms.

RECENT ACCOMPLISHMENTS

Normal and Abnormal Development

Research in normal and abnormal development seeks to define the events that occur during the development of the visual processing system. The ultimate goal is to be able to explain the mechanisms by which such development occurs, including identification of the role of genetics, experience, and interactions in normal and abnormal development. Essential to this goal is a thorough understanding of the events of development at the molecular level. Accordingly, molecular research on the development and function of the central visual processing system was given major emphasis in Vision Research--A National Plan: 1983-1987. An NIH program announcement encouraging the submission of research project grant applications for high-quality molecular research in all areas of strabismus, amblyopia, and visual processing was published in March 1984. This emphasis is timely, due both to recent developments in methodology for molecular research and also to the groundwork that has been laid by physiological and anatomical research. Some of the recent findings in molecular studies of the developing or regenerating visual system are given below.

Molecular Studies. Molecular factors that stimulate, regulate, or guide neurite growth in the visual system are the focus of active research efforts. These studies provide key information regarding how and why neurite growth occurs, normally or abnormally. Cell cultures are being

used for much of this work, and one approach has been to study the appearance during neurite development of particular components that presumably are related to the development and/or functioning of the visual system. For example, Adler[1] is studying the appearance during neurite development of specific uptake systems for taurine and gamma aminobutyric acid (GABA). He has identified a specific taurine uptake system that requires sodium, is temperature dependent, and is inhibited by substances such as GABA. While GABA most likely functions as an inhibitory neurotransmitter, the precise function of taurine is not yet clear. Taurine does have inhibitory neurotransmitter activity, but many investigators now believe that these effects are not physiologically important. What is known is that taurine is found in large quantities both in the retina and the brain and that a majority of neurons examined have specific taurine uptake systems. Taurine does seem to be important for photoreceptor survival and may play other essential roles in the visual system; however, what those roles are must yet be discovered.

High affinity GABA receptors were found to appear in developing chick neural retinas on embryonic day 10. In other experiments designed to set the stage for identifying those factors that determine when the GABA receptors appear, neuronal cultures were prepared from 8-day-old embryos and examined for high-affinity GABA receptors after various days in cell culture.[2] The appearance of these receptors in culture followed a time course that is reasonably similar to what is observed for in vivo development; this finding will make it possible to study the appearance and function of GABA receptors in a relatively simple system.

Adler is also studying the effect on neurite growth of the surface characteristics on which the neurons are growing. He found that chick neural retinal cells are able to attach to polyornithine-coated dishes in serum-free medium supplemented with a neuronal extract, but they do not produce neurites under these conditions. However, when the substratum is pretreated with either laminin or conditioned medium containing PNPF, neurites sprouted.[3] The results suggest that either laminin, PNPF, or a related substance provides a signal necessary for neurite sprouting during normal in vivo development.

Liem and others are studying the appearance during development of each of the triplet polypeptides that make up the neurofilaments in optic nerves of rats.[4] Of the three peptides, only the 150,000 molecular weight neurofilament protein was present at postnatal age day one. The 68,000 molecular weight protein was first detected on the sixth day, at about the same time as the appearance of the neurofilamental structures. However, the 200,000 molecular weight protein was not seen until day 20.

While the studies cited above identify when specific macromolecules appear in developing systems, other types of studies are needed to determine precisely how the mature macromolecules are synthesized and/or modified, as well as how their synthesis is regulated. In studies of the regulation of synthesis and the post-translational modification of neurofilament proteins, Willard has developed a system for the in vitro translation of the messages for M and L proteins.[5] His results lead him to

conclude that the post-translational modification observed for the H protein, attributed by others to be due to phosphorylation, is probably actually due to proteolysis.

GAP-43 is a growth-associated protein found in enriched levels in neuronal growth cones and therefore probably is involved in some way in the growth of the neurites. Willard has shown that GAP-43 can be phosphorylated.[5] Such phosphorylation is stimulated greatly by the presence of calmodulin and calcium. Thus, the possibility exists that GAP-43 is a protein kinase, or at least a protein related to the phosphorylating system.

Much attention has centered on the role of a cell adhesion molecule isolated from nerve tissue, known as N-CAM, and its possible role in the formation of visual pathways. Studies on this glycoprotein, some of which involve the use of antibodies, strongly suggest that it guides the development of visual axons such as in the optic nerve. Research in the future is likely to focus increasingly on biochemical approaches, as well as more refined morphological analyses, to characterize the development of the visual system. Monoclonal antibodies now make it possible to localize specific proteins in tissue sections and to perform structural characterizations with much greater resolution than before. For example, studies with monoclonal antibodies show that an intermediate filament-associated protein known as p50 is localized periodically on the filament,[6] whereas vimentin is uniformly distributed along the filament.[7]

Relative quantities of peptides in a tissue extract can be estimated by the relative intensities of staining of bands or spots after gel electrophoresis; incorporation of radioactive amino acids is used to determine relative rates of peptide synthesis and can be used to determine the cell type, tissue, or organ in which a given peptide is synthesized. This approach is being used to study the events that occur during optic nerve regeneration. The rationale for such studies is that by studying regeneration in organisms such as goldfish that are capable of this process, we might learn to stimulate nerve regeneration in humans. Schechter isolated the optic nerve, the optic tectum, and the retina of goldfish and incubated them separately in the presence of radioactive methionine.[8,9] The peptides were then analyzed by electrophoresis. Four peptides were the focus of his studies. All four were found in the optic nerve at about equal concentrations, but only two were radiolabeled when isolated optic nerves were incubated in radioactive methionine. Initial results suggest that these two proteins are synthesized by nonneuronal cells, quite possibly glial cells. The other two peptides occur in the retina, but only in small quantity. However, they become highly radiolabeled when the retina is incubated with radioactive methionine, suggesting that they normally are synthesized in the retina and then transported to the optic nerve.[10]

Gordon-Lickey, Daw, Kasamatsu, and others are studying the role of catecholamines in plasticity (ability to change) of the visual system. For approximately five years, investigators have been intrigued by results, originally obtained by Kasamatsu and Pettigrew,[11-13] suggesting that depletion of norepinephrine by administering 6-hydroxydopamine (6-OHDA) preserves the ability of a monocularly deprived eye in kittens to drive

electrophysiologically the cells in the visual cortex and prevents the shift from binocularity that normally occurs after monocular deprivation. However, the situation has been confused by the finding that the means by which 6-OHDA was administered determines whether or not plasticity is affected.[14,15] Daw[16] has extended these findings to show that 6-OHDA also prevents the cortical effects that kittens normally undergo when they are reared in an environment continually moving past them in one direction only. Lowering the epinephrine level via direct cortical injection did affect plasticity, but four other procedures that depleted norepinephrine levels by the same extent did not affect plasticity. In research to examine further the effect of 6-OHDA on plasticity, Gordon-Lickey has used the very sensitive method of high pressure liquid chromatography to measure norepinephrine levels in the brains of kittens after 6-OHDA treatment.[15] She finds that doses as low as 0.2 mg of 6-OHDA depletes the norepinephrine level by 90 percent, but much higher doses apparently are required to produce the observed behavioral and electrophysiological effects on plasticity. This finding raises the possibility that 6-OHDA exerts its effect on plasticity via some other mechanism, rather than through the lowering of the norepinephrine level.

Cell and Systems. Research in this area has focused on the study of mechanisms by which developing cells and their processes assume the highly organized architecture which characterizes the visual system.

In an example of the use of an abnormally developing system to study both normal and abnormal development, Pearlman[17] and his colleagues are using the mutant "reeler" mouse as a model to study the formation of connections between cells in the developing visual cortex. In adult reeler mice, cortical neurons are not located in their appropriate positions, yet they do make appropriate connections with other neurons. Programmed cell death is known to be a normal feature of mammalian embryonic nervous system development. However, close examination of the development of embryonic reeler mice revealed that the location of dying cells is abnormal. These findings seem to minimize the importance of cell location in the formation of appropriate connections but do point to the need for further studies in the regulation of neural cell death. Other workers have concentrated their efforts on the mechanisms which control the establishment of connections between developing centers of the visual system in normal animals. Shatz and her colleagues[18] have completed a study of the development of projections between the retina and lateral geniculate nucleus (LGN) in the embryonic cat. They showed that the geniculocortical projections extend to just beneath the developing cortex by the 35th embryonic day, where they undergo a waiting period before invading the cortex. Shatz and co-workers currently are investigating factors that regulate this waiting period. In related work on synaptogenesis in the cat LGN, they have determined that the well-known adult pattern of synapses in the LGN emerges at the 39th embryonic day. Electrophysiological studies with in vitro slice preparations are being used to determine when developing synaptic complexes respond to visual stimuli.

Research with lower vertebrates can provide clues to the mechanisms of plasticity and how the visual system changes during development. Udin[19] is characterizing the morphological changes that axons projecting to the optic tectum undergo during metamorphosis of the amphibian Xenopus from tadpole

to frog. During metamorphosis, the animal's eye migrates from the side to the front of the head and binocular vision develops. Udin has found that axonal arbors occur over a greater proportion of the optic tectum as eye migration proceeds, while other branches are resorbed.

Behavioral Studies. Accumulating evidence points to several important events in human visual development that occur around the fourth postnatal month. Earlier studies by several investigators showed that stereopsis is initiated in human infants at an average age of four months; this is also the age at which strabismic amblyopia may begin. Recent studies show that binocular summation of the pupillary response begins at about the same age, as does infants' subjective preference for binocularly fused stimuli,[20] as opposed to rivalrous stimuli.[21] Vernier acuity begins to be better than grating acuity at about the fourth month.[22] It is interesting to note that while grating acuity develops at about the same rate for boy and girl babies, both vernier acuity and stereopsis seem to develop faster in girl babies, at least during the age range of 4-6 months.[23]

Recent sensitive studies of the development of grating acuity reveals a developmental plateau in human infants beginning about age five months.[24] This plateau lasts for several months, after which the grating acuity resumes its upward trend.

Structure and Function

Research on the structure and function of the visual processing system remains a very active area. Research at the molecular level is still very limited because of the difficulty of identifying research problems in the structure and function of the visual system that are amenable to molecular approaches. As it stands, many molecular approaches, such as neurotransmitter identification or the use of monoclonal antibodies to identify specific components, must by necessity be combined with anatomical or neurophysiological approaches because of the complexity of the visual system.

Cell and Systems. Much of the progress in the anatomical and physiological study of neuronal circuits in the central visual system has hinged on the application of increasingly precise neuronal marking techniques, including those which morphologically label cells after they have been characterized physiologically. A number of laboratories continue their work in tracing the central visual processing pathways, and invertebrates often have served as useful models in which to learn some of the anatomical bases for complex physiological interactions. Fahrenbach[25] has completed an anatomical reconstruction of all the synapses in the neuropile (a plexus of neural processes) in the compound eye of the widely studied invertebrate, Limulus. He has identified the anatomical features underlying the inhibitory effects known to occur between components within the eye of Limulus and has shown that the inhibitory processes are literally hard-wired into the anatomical plexus. He found that the strength of the inhibition varies at different points in the neuropile and can be correlated with the density of synapses in this structure. From his data, Fahrenbach can predict the source and the strength of the inhibition from the structural analysis. These findings are likely to guide the way

for anatomical studies of physiologically characterized neural processes and synaptic patterns in more complex neural structures, such as those found in mammals.

A number of investigators have traced projections from retinal X-, Y- and W- cell pathways in mammals. This approach often has yielded elegant results but, due to its laboriousness, has characterized only a small sample of the intricate neural interactions. Spear and his colleagues[26] have introduced a novel approach to this problem. They have prepared an antibody which preferentially produces lesions in the large retinal ganglion cells (Y-cells) after intraocular injection into the eye of the cat. By injecting high doses of the antibody into one eye, they have been able to lesion selectively the Y-cell input of that eye to the superior colliculus. These studies have interesting implications: they suggest that the superior colliculus' control of orienting responses to visual stimuli are mediated by W-cell input. Antibody approaches, such as the one used here, are likely to replace the use in many instances of invasive electrolytic, chemical, or mechanical lesion techiques for identifying the functions of specific cell populations.

Behavioral Studies. Much behavioral research today is aimed at understanding better how the visual system functions. This approach has the advantage that, due to its noninvasive nature, it can readily be applied to humans. Bodis-Wollner[27] has developed a system for studying binocular interactions that involves a haploscopic arrangement of two CRTs such that one eye views a steady pattern on one CRT and the other eye views a counterphase modulated pattern on the other CRT. With this arrangement, Bodis-Wollner finds no appreciable first harmonic, which is in contrast to the situation seen for monocular addition. This implies that the first harmonic signature seen for monocular addition originates prior to binocular interaction.

Many investigators are seeking to adapt the findings of behavioral research to clinical purposes. The use of visual evoked potentials has been thought by many to be an objective procedure that is applicable to a variety of clinical conditions, such as multiple sclerosis and amblyopia, and which can be used to measure the visual characteristics of persons too young or otherwise unable or unwilling to cooperate. To be useful as a clinical tool, any procedure must produce reproducible, reliable results. There have been suggestions that different electrode sites for measuring visual evoked potentials would produce different linear and nonlinear properties and/or different frequency responses. However, studies by Bodis-Wollner[27] with normal subjects indicate that different electrode placements do not produce differences in frequency composition. One does obtain signals of varying amplitude, with the largest and most consistent in-phase steady state signals recorded around the inion, at the back of the head.

Results by Enoch and others[28] have indicated that hyperacuity thresholds are relatively insensitive to retinal image degradation and thus might be useful clinically for assessing visual function behind ocular opacities. Such determinations would help the surgeon predict whether or not vision is likely to be restored by cataract removal. Enoch is developing a combination of two tests for assessing visual function behind opacities. One test measures the patient's ability to align two small spots of light as a function of the gap separating the spots. The other, called hyperacuity perimetry, measures the patient's ability to align two spots of light separated by a gap of 16 min. arc while fixating different visual field locations.[29-31] The two tests have been applied to selected patients and appear to have good predictive value of visual function to be expected after cataract removal.

Amblyopia

Current research on amblyopia is directed towards characterizing the nature of the visual sensory deficit, determining the mechanism and conditions under which the deficit occurs, and devising improved therapies for treatment.

Flom has been measuring spatial imprecision (uncertainty) and inaccuracy (distortion) in strabismus and amblyopia, as determined by subjective judgements of the horizontal position of a flashed vertical test line relative to the vertical axis of a reference target.[32] Spatial aberrations seen in strabismics could not be mimicked by reducing the acuity of normal eyes by optical blur or by decreasing photopic luminance. In fact, many strabismics with normal or nearly normal acuity still exhibited spatial imprecision and, in some cases, substantial inaccuracy.[33] Flom concluded that the spatial imprecision and inaccuracy did not seem to be caused by reduced visual acuity; rather, the spatial aberration, especially the spatial imprecision, probably produces visual acuity deficits.[34] The effects seemed to be correlated with the severity of the spatial aberration.

On the other hand, for a given reduction in visual acuity, anisometropic amblyopes' spatial imprecision was much less than that of the strabismics; anisometropes with nearly normal visual acuity have essentially normal spatial precision. Thus, spatial abnormalities seem to be the primary deficit in strabismic amblyopia, while impaired resolution may be the primary deficit in anisometropic amblyopia.

Movshon and co-workers have investigated the spatial properties of cortical neurons in area 17 of macaque monkeys that had been made surgically esotropic shortly after birth.[35] They found that the monkeys had undergone a profound loss of spatial resolution and contrast sensitivity in neurons driven by the deviating eye, as well as a marked ocular dominance shift in favor of the non-deviating eye and a dramatic loss of cortical binocularity. Their results differ dramatically from those obtained earlier in cats which, after they are made surgically esotropic, normally lose binocularity but undergo no change in spatial sensitivity. These differences may be due to different procedures used in

cats and monkeys for surgically producing strabismus, or they may reflect significant interspecies differences in cats' and monkeys' responses to strabismus.

Ocular Motility and Strabismus

Conjugate Eye Movements. A key to the precise study of eye movements is, of course, the ability to measure these movements very accurately. Measuring vertical eye movements has been particularly difficult because of the occurrence of eyelid movements. In a comparison of electro-oculography, infrared, and search coil methods, preliminary data by Baloh[36] and Yee[37] found the latter procedure to be least affected by eyelid movements. Artifacts were minimized but not eliminated if the upper eyelid was immobilized. Furthermore, both of these investigators, as well as several others are developing eye movement techniques as clinically useful tools.

Noda has used microstimulation (3-8 microamperes) of the primate flocculus to generate eye movements.[38,39] He observed greater variation in eye movements than had been seen by others with larger stimulating currents. Several different types of eye movements in several different directions were produced by stimulating different points. Peak eye velocity increased with increasing stimulus intensity, and the velocity peak occurred earlier. When thresholds were averaged for sites where stimulation did produce eye movements, the mean threshold was highest in the Purkinje layer, intermediate in the molecular layer, and lowest in the granular layer. These results suggest a means of analyzing in a more precise manner the anatomical and functional aspects of eye movements and of the regions of the brain that are involved in these eye movements.

In saccadic eye movements, burst neurons provide the phasic drive for the motor neurons, while the omnipause neurons, which are located in the midline pontine region, suppress saccades during intersaccadic intervals. A big question has been the origin of the signal to the omnipause neurons. Fuchs and co-workers[40,41] have identified brainstem afferents to the omnipause region by retrograde transport of horseradish peroxidase, which was injected into the region where omnipause activity had been recorded. A number of specific brainstem nuclei were labeled, with the results suggesting that the initiation signal for the omnipause neurons most likely comes from the mesodiencephalic reticular formation and/or the superior colliculus.

Eye movements can be elicited in monkeys by stimulating either one of two general cortical areas. One is the anterior area, known as the frontal eye field, which seems to project directly to the oculomotor core in the brainstem. The other is a posterior area, including the striate, prestriate, and inferior parietal cortex, which projects to the superior colliculus and from there to the oculomotor core. Keating[42] found that destroying the superior colliculus eliminates eye movements in response to electrical stimulation of the posterior group but does not eliminate eye movements in response to electrical stimulation of the frontal eye field. Conversely, removing the frontal eye fields and the inferior parietal cortex did not eliminate eye movements produced by stimulation to the striate cortex.[43]

A very exciting area of research at present is that of multisensory integration, that is, how inputs from the various senses affect responses to each other. Sparks has been studying interactions between visual and auditory stimuli. Monkeys were trained to look to either visual or auditory targets in the dark, and receptive fields of sound-sensitive cells in the superior colliculus were plotted as a function of direction of visual fixation. For every sound-sensitive cell encountered, the position of the eyes in the orbit affected the neural response to the auditory stimulus.[44-46] The magnitude of the auditory neurons' discharge was determined both by the position of the auditory stimulus and by the position of the eyes in the orbit. Thus, there seems to be a system for signalling a motor error for the difference between the actual and desired eye position, which uses both auditory and visual input.

In other studies of the neuroanatomy of eye movements, Sparks is characterizing neurons in the mesencephalic reticular formation that discharge prior to saccades. Microstimulation of these neurons produces saccades,[47] indicating their role in the generation of this type of eye movement. The neurons have collicular-like movement fields, such that stimulation along a single penetration produces different effects at different depths. At superficial sites, the size and direction of saccades are largely independent of stimulation parameters and of eye position in the orbit; but at deeper sites, the orbital position during fixation influences the size and/or direction of the saccade.

Vergence and Accommodation. Research on vergence and accommodation is important because little is known about the underlying systems, and disorders affecting vergence and accommodation are common. Furthermore, research on vergence and accommodation is needed because of the possible importance of these processes to other conditions, such as refractive error. In spite of the importance of this area, past progress has been limited, at least partly because of the difficulty of such research and also because of the existence of only a limited research base. However, recent findings and new approaches in related areas have enhanced research opportunities in vergence and accommodation. An NIH program announcement was published in March 1984, encouraging the submission of research project grant applications in vergence eye movements. Some recent findings in research in vergence and accommodation follow.

Owens found that intermediate spatial frequencies (3-5 cycles/degree) are more accurate stimuli for accommodation than are either higher or lower spatial frequencies.[48] Furthermore, contrast exceeding the psychophysical threshold by at least one log unit is needed to induce accommodation.

Earlier studies showed that even short periods of reading produce striking changes in vergence and accommodation. Owens has extended these studies to include examination of the effects of monocular reading with the other eye patched. Monocular reading induces a myopic shift of the dark focus and accommodative response of the unpatched eye but no apparent induction of accommodation.[49] However, monocular reading induces a more divergent change in vergence and distance phoria than occurs after binocular reading. These results provide strong evidence for dissociated mechanisms regulating vergence and accommodation.

Electrophysiological study of vergence eye movements has resulted in the localization by Mays of cells with either a pure vergence velocity signal or a mixed vergence velocity and vergence position signal.[50] In more recent research, he finds that prism or convergence adaptation produces adapative alterations in the firing rate of convergence cells: most fire at a lower rate after adaptation, and the slope of the rate-vergence angle curve changes for some cells but not for others.[51] These results imply that, in addition to the rapid vergence mechanism, there is a slowly adaptable tonic vergence system. Mays is attempting to identify cells of the latter type.

Fusional eye movements can be elicited by disparities in small portions of the peripheral visual field. Kertesz has used both subjective and objective measurements to characterize the fusional response.[52] He finds that the motor response is insufficient to account for all of the fusion response and suggests that fusion must involve some nonmotor components as well.[53] The composition of the fusional response seemed to be a function of the eccentricity and of the angular position of the stimulus; the motor compensation was proportionally larger when the stimulus disparities were positioned close to the line of sight or along the superior visual meridian.

In view of the widespread nature of vergence anomalies, many investigators are seeking therapeutic means of correcting these problems. Kertesz[52] found that therapy with wide-angle fusional stimulation was helpful to 11 of 13 patients with convergence insufficiency and to 12 of 15 strabismics with fusion and normal retinal correspondence.

Erichsen[54] has been studying the regulation of pupil size and accommodation in pigeons. He finds that stimulation of the Edinger-Westphal nucleus produces both accommodative changes and pupillary constriction, while stimulation of the area pretectalis produces pupillary constriction but no accommodative change. Injections of HRP were used to label retrogradely retinal ganglion cells that project to the area pretectalis. These studies show that the area pretectalis receives input from a morphologically distinct subpopulation of retinal ganglion cells that constitutes only a small percentage of the total retinal ganglion population. These cells are distributed nonuniformly across the retina. Furthermore, projection from the pretectum to the Edinger-Westphal nucleus is entirely crossed, not partially uncrossed as was previously thought.

Specific neurotransmitters no doubt function in pupillary responses and in accommodation. In recent studies, Erichsen has found enkephalin and substance P in ganglion cell endings known to innervate the iris and ciliary[55] body. Although the motor changes in this pathway are thought to be effected via acetylcholine, as is the case in other contractile tissue, the enkephalin and substance P in these nerve endings may help to modulate synaptic transmission.

Riggs is performing psychophysical research to elucidate the effects of eye blinks and eye movements on visual processing. He finds that both reflex and voluntary eye blinks cause visual suppression.[56,57] Furthermore, both convergent and divergent eye movements are also accompanied by visual suppression.[58] Thus, there seems to be a general association between

visual suppression and eye motor activity.

Muscle Physiology. Chiarandini and co-workers are investigating multiply innervated fibers in the global and orbital layers of the extraocular muscles. Several differences have been found in the multiply innervated fibers of the two layers.[59] Some of these differences are: orbital multiply innervated fibers contain only four to ten nerve endings,[60] whereas global multiply innervated fibers may contain thirty to fifty endings. A monoclonal antibody known as ALD-180, derived from the slow ALD (anterior latissimus dorsi) muscle of chickens, selectively reacts with the distal portion of the orbital multiply innervated fibers but does not react with the middle region of these multiply innervated fibers, or at all with fibers in the other layer. Multiply innervated fibers also differ in their ATPase activity: orbital multiply innervated fibers contain both acid and alkaline ATPase, whereas global multiply innervated fibers reveal only acid ATPase activity. These differences are consistent with a role of global muscles in tonic tension and a more heterogeneous role for orbital multiply innervated fibers.

Breinin has been collaborating for some time with Chiarandini in a careful characterization of extraocular muscles in rabbits and rats. In view of the structural and functional variations that were found to exist along the length of multiply innervated muscle fibers, Breinin is now performing a careful study of white fibers, which are singly innervated and account for most of the muscle volume in the global region. Breinin[61] has found some morphological differences in myofibrillar organization in the middle and distal portions of the global region but not as much variation as has been observed with the multiply innervated fibers.

Strabismus

When other means of treating esotropia are unsuccessful in maintaining alignment of the eyes, surgical intervention becomes necessary. Unfortunately, a single session of surgery often is insufficient to produce good eye alignment; there is good evidence that only about 50 percent of patients with acquired esotropia achieve good eye alignment with one operation, and second, third, or even more operations often are necessary.[62] The reasons for this need for additional surgery are unclear. However, preliminary data suggest that the preoperative use of prisms may result in good eye alignment with only one operation each for approximately 85 percent of the patients that undergo this treatment. The procedure makes use of the observation that many patients respond to the fitting of prisms by altering their alignment so that a new, stable interocular angle is achieved.

A multicenter, randomized, prospective clinical trial was begun in March 1984, to determine whether prism adaptation is a useful adjunct to surgery to correct acquired esotropia. The specific goals of the clinical trial are to assess: (1) the overall beneficial effect of the prism adaptation procedure by comparing patients who respond to prism adaptation by developing a new stable angle of deviation and who have surgery for the prism-adapted angle, responders who have surgery for the original angle, non-responders to the prisms who have surgery for the original angle of deviation, and patients who do not have the prism adaptation test and have

surgery for the original angle of deviation; (2) whether patients with acquired esotropia who respond to prism adaptation are more accurately corrected by operating for the prism-adapted angle or the original angle of deviation; (3) whether prism adaptation responders have a better surgical result than patients who do not respond to prism adaptation; and (4) whether certain input variables are useful in predicting which patients are more likely to benefit from prism adaptation.

Neuro-ophthalmology. Zee and co-workers have obtained evidence for considerable plastic, adaptive change in the smooth pursuit system.[63-65] This capacity for adaptive change may signal a heretofore unrecognized potential for rehabilitation in visuomotor coordination in patients with damage to the cerebral cortex. These investigators also have developed a model of the neural gaze-holding networks that combines position and velocity feedback and which can be used to explain most of the abnormal eye movements seen in patients with congenital nystagmus.

Visual Impairment and Its Rehabilitation

Research in this field has focused on characterizing the nature of the residual vision in patients with permanently impaired vision from a variety of etiologies and on developing and evaluating aids for visually impaired people. One project is investigating eye movements in patients with central visual field scotomata and in normal subjects with simulated central scotomata.[66] The investigators have found that virtually all patients with bilateral scotomata of over three years' duration adopt a strategy of eccentric fixation with a preferred eccentric viewing angle that positions the image of a visual target in a region outside the scotoma, thereby making the image visible. Considerable individual differences were observed in the direction of preferred gaze, variability of eye position, eye drift direction, and velocity. The eyes of many patients with macular disease show a tendency to drift toward a straight ahead position, thus losing the target intermittently in the central scotoma. Normal subjects with simulated scotomata tended to drift in a particular direction, but the drift direction varied across subjects and was never observed to be consistently directed toward the central scotoma. Another interesting finding in this study was that subjects with macular scotomata who fixate to the left of their visual field defects exhibit a highly consistent decrease in reading rate and accuracy when compared with subjects with similar scotomata but different viewing angles.

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BIOMETRY AND EPIDEMIOLOGY PROGRAM

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE ASSOCIATE DIRECTOR FOR BIOMETRY AND EPIDEMIOLOGY

Fred Ederer

Organization

Under a reorganization of the National Eye Institute, the Office of Biometry and Epidemiology has become the Biometry and Epidemiology Program, National Eye Institute, with Mr. Fred Ederer serving as Associate Director for Biometry and Epidemiology and Dr. Daniel Seigel as Deputy Associate Director. There are two Branches, Clinical Trials which is headed by Dr. Seigel, and Epidemiology which is headed by Mr. Ederer, and two Sections, Biometry which is headed by Dr. Roy Milton and Medical Statistics which is headed by Dr. Sylvan Green.

Functions

The Biometry and Epidemiology Program has three main functions: research, education, and consultation.

Research is the dominant function. It is the Program's mission to plan, develop, and carry out human population studies concerned with the causation, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures.

Education: The BEP carries out a program of education in biometric and epidemiologic principles and methods for the vision research community. This function consists of courses, workshops, a fellowship program for ophthalmologists, publications, and consultation and collaboration on research.

Consultation: The Program provides biometric and epidemiologic assistance to National Eye Institute intramural and extramural staff and to vision research workers elsewhere. The assistance ranges from referral to appropriate consultants through collaboration as co-investigator.

Research Activities

Clinical Trials. Two contract-supported, randomized multicenter clinical trials on the treatment of diabetic retinopathy are in progress. These are the Early Treatment Diabetic Retinopathy Study (ETDRS) and the Diabetic Retinopathy Vitrectomy Study (DRVS).

The ETDRS was designed to provide a better understanding of the best time to use photocoagulation in the course of diabetic retinopathy.

Patients with macular edema, preproliferative retinopathy, and mild or moderate proliferative retinopathy are being studied. Three forms of photocoagulation treatment, ranging from restricted focal treatment to complete panretinal photocoagulation, are being compared with no photocoagulation. In addition, the study is evaluating the effect of daily administration of aspirin, in a comparison with placebo controls, on the incidence of microvascular and macrovascular complications. The study is also investigating factors associated with the progression of disease. As of July 15, 1984, 3,662 treatment allocations had been issued, and 3,315 patients had been treated. Recruitment will end in March 1985 with about 4,000 patients enrolled in the study. Drs. Lloyd Aiello and Frederick L. Ferris, III serve as Co-Chairmen for the ETDRS and Dr. Richard L. Mowery as Project Officer.

The DRVS has succeeded in recruiting a group of patients having a total of 997 eyes eligible for the study: 616 eyes with vision reduced by hemorrhage into the vitreous (group H) and 381 eyes still having useful vision but with serious risk of complications that often lead to retinal detachment (group NR). Recruitment terminated in June 1983. Half of the eligible eyes in each group were randomized to prompt vitrectomy; in group H the other half was randomized to vitrectomy one year after the hemorrhage, if treatment was still indicated; in group NR the other half was randomized to "traditional" care. The results of the first two years' experience in the H group is being prepared for publication. A manuscript summarizing the first two years of the natural history component of the study has been submitted for publication.

Dr. Robert Sperduto has been active in the scientific management of a grant-supported clinical trial, the Prospective Evaluation of Radial Keratotomy Study (PERK), which is designed to evaluate a surgical procedure--radial keratotomy--to correct myopia. One-year data will be presented at the American Academy of Ophthalmology in November 1984. A publication will follow in December 1984.

The Clinical Trials Branch implemented the Krypton-Argon Regression of Neovascularization Study (KARNS) in three pilot clinics in December 1983 to test the examination procedures and data collection forms. The major objective of this randomized clinical trial is to compare krypton laser to argon laser panretinal photocoagulation for treating neovascularization on the optic nerve head caused by diabetic retinopathy. The pilot phase was successfully completed in June 1984 and new clinics were enrolled in KARNS starting in August 1984. It is expected that by March 1985 a total of at least 35 clinical centers will be actively recruiting and randomizing patients for the study. As of September 1, 1984, 32 patients had been randomized. This study is unique for the National Eye Institute since the functions for both the coordinating center and the fundus photography reading center are being handled by staff of the Clincal Trials Branch. Another unique feature of this multicenter trial is that the participating clinics receive no financial reimbursement from the National Eye Institute for their participation. If successful, this model may facilitate the conduct of some future clinical trials in eye diseases. Dr. Ferris serves as Co-Chairman for this study. Dr. Mowery serves as Director of the Coordinating Center.

Patient follow-up in the Diabetic Retinopathy Study (DRS) was terminated in 1979. The study's data base has been organized in a fashion that is usable by any interested investigator, and is now available from the National Technical Information Service. A methodological paper on the early stopping of a clinical trial and an epidemiologic paper assessing risk factors for visual loss in the DRS have been submitted for publication. A final results paper and a paper on mortality in the DRS patients are in the final stages of preparation. A paper written by Mr. Ederer and Mr. Marvin G. Podgor on early stopping of a clinical trial, using the stopping of the DRS as a case study, has been accepted for publication by the Journal of Controlled Clinical Trials.

Dr. Seigel is serving as project officer for a randomized trial of sorbinil, a drug manufactured by Pfizer Laboratories. The drug is an aldose reductase inhibitor and has potential for preventing or retarding diabetic neuropathy and retinopathy. The NEI is providing scientific leadership for this multiclinic trial, which is funded by Pfizer. As of August 15, 1984, 184 patients had been randomized to treatment. Recruiting has been slower than expected and alternative design proposals are being discussed to ensure adequate statistical power at the end of recruitment and follow-up.

Dr. Mowery was named Project Officer for the Early Treatment Diabetic Retinopathy Study (ETDRS) in March 1984. In this position he is responsible for the administrative coordination of the study's twenty-three clinical centers, the Coordinating Center, and the Fundus Photograph Reading Center. He serves as a member of the Operations Committee, Executive Committee, and Analysis Planning Group.

Dr. Green served as Study Statistician for the randomized clinical trials of the National Cancer Institute's Brain Tumor Cooperative Group, including study design, data collection, monitoring, and analysis.

Dr. Green analyzed the data for the NCI Intergroup Testicular Cancer Study, involving investigation of prognostic factors and the role of adjuvant therapy.

Epidemiology. The final report on the pilot study of the Visual Acuity Impairment Survey (VAIS) was prepared in January 1984. The report indicates that home screening of visual acuity with pinhole correction by Census Bureau lay interviewers could correctly identify nearly all persons whose acuity is 20/50 or worse. Other aspects of the pilot study demonstrated the feasibility of the design, but the one critical difficulty was the small percentages of both positive and negative persons screened who participated in the clinic examination, 52% and 54%, respectively. The clinic participation rate was strongly and inversely related to age. The major recommendation from the report regarding the feasibility of a national VAIS was for the Office of Biometry and Epidemiology to pursue the feasibility of collaborating with the National Center for Health Statistics in the 1987 Health and Nutrition Examination Survey. Meetings of the two staffs have been held, and planning for a collaborative effort is in progress.

A VAIS Publication and Editorial Committee was established in August 1983 to identify possible manuscripts to be prepared from the pilot study data

and to review manuscripts prior to submission for publication. Five manuscripts were identified and writing teams selected. Two manuscripts have been completed and submitted, one to the Archives of Ophthalmology and one to the Journal of the American Public Health Association. Mr. Ederer chaired the VAIS Publication and Editorial Committee, Dr. Mowery served as Executive Secretary, and Mr. Dean Krueger was a member of the committee.

Several staff persons are planning the second symposium on eye disease epidemiology, to be held June 5-7, 1985. Topics that will be features are epidemiology, methodologic issues in clinical trials, statistical methods for analysis of eye data, natural history studies, and diagnostic methods of measurement and classification. Dr. Seigel is Chairman for the planning committee.

An RFP has been published inviting clinics to participate in a multicenter case-control study of selected eye diseases. This case-control study would complement current or previous ones on cataract, senile macular disease, and diabetic retinopathy. Two clusters of eye diseases are being considered for study. In the first, cardiovascular risk factors are of particular interest. The second cluster consists of selected types of uveitis. Data management for this study will be done through contract; data analysis will be done within the BEP. Dr. Robert D. Sperduto and Dr. Seigel are Co-Chairmen of this project and Dr. Mowery serves as Project Officer.

Mr. Podgor and Dr. Gary Cassel of the Wilmer Institute have been investigating associations of survival and lens changes in the Framingham Eye Study population. Mr. Podgor presented some of their results at the 1984 ARVO meeting. A manuscript is nearing completion.

Dr. Sperduto collaborated with Dr. M. Cristina Leske of the State University of New York at Stony Brook in the preparation of a grant application for a case-control study of senile cataract.

Dr. Sperduto and Dr. Milton visited New Delhi, India, in September 1983 for final development of a protocol for a collaborative case-control study of senile cataract, sponsored by the Indian Council of Medical Research and under the US-Indo Science and Technology Initiative for cooperation in research. Dr. Sperduto is Co-Principal Investigator for this study, which received final approval in October, and a manual of procedures has been developed which should be implemented this fall.

While in India in September 1983, Dr. Milton and Dr. Barbara Underwood visited current and potential collaborating Indian research centers in Hyderabad, Madurai, and Chandigarh. In Hyderabad, Dr. Milton participated in monitoring and reviewing of a study of the relation between measles and keratomalacia.

Dr. Milton and Dr. Seigel participated in a US-Japan Workshop on Behcet's Disease, sponsored by the Japan Society for Promotion of Science, the NEI, and Sandoz-Japan, in which a protocol was developed for a double-masked clinical investigation of cyclosporine in Behcet's disease with visual disorder.

Dr. Sperduto and Mrs. Rita Hiller used data from the Framingham Eye Study to estimate the prevalence rates of nuclear, cortical, and posterior subcapsular lens opacities in persons between ages 52 and 85.

Dr. Sperduto and Dr. Ferris collaborated with others in the publication of a paper that demonstrated the effect of asymmetrical senile macular degeneration on the visual acuity of a trained artist.

Dr. Sperduto prepared a chapter on cataract for a WHO publication on research priorities for the prevention of blindness in developing countries.

Education

Dr. Carl Kupfer, Mr. Ederer, Dr. Ferris, Dr. Seigel, and Dr. Sperduto participated as faculty in the fifth of a series of annual courses on epidemiologic and biostatistical approaches to clinical vision research. Along with three university colleagues, Drs. Theodore Colton, Matthew Davis, and Charles Hennekens, they presented a three-day course in Sarasota, Florida, for clinical investigators just before the 1984 ARVO annual meeting. The course was attended by about seventy people from academic institutions and, according to written evaluations, was well received by them. Plans are under way for a sixth course in 1985.

Dr. Ferris and university colleagues taught a course on Clinical Guidelines for Photocoagulation and Diabetic Retinopathy at the annual meeting of the American Academy of Ophthalmology, November 1983.

During 1984, Dr. Ferris taught courses at the Wilmer Ophthalmological Institute at Johns Hopkins University in Baltimore in diabetic retinopathy and macular degeneration, and participated in a symposium on clinical research at the American Association of Pediatric Ophthalmology and Strabismus meeting. He also presented papers on the current treatment of diabetic retinopathy at the Florida Society of Ophthalmologists meeting.

Eight lectures for the NEI Clinical Branch on Clinical Research Methods were given by Dr. Ferris, Mr. Ederer, Dr. Seigel, and Dr. Sperduto.

Dr. Green presented a series of lectures on medical statistics to medical students participating in the NIH course on Computers in Clinical Medicine.

Collaboration and Consultation

Dr. Mowery worked with the NEI Director's and Deputy Director's offices in developing a proposal on operations research in cataract surgery in India. The proposal outlined a program for evaluating the effectiveness of four methods for encouraging persons with cataracts to obtain medical treatment. Dr. Mowery is the Project Officer for this study.

Dr. Mowery provided consultation to Drs. Anita Suran, Jack McLaughlin, and Israel Goldberg of the Extramural and Collaborative Programs Branch, NEI, on the design and development of clinical trials in glaucoma and retinitis pigmentosa.

Dr. Mowery consulted with Dr. Basil Rifkind, Chief of the Lipid Metabolism-Atherogenesis Branch, National Heart, Lung and Blood Institute, on the development of a follow-up study of men and women screened in Moscow and Leningrad as part of the Joint US-USSR Collaboration.

Dr. Mowery reviewed for the NIH Division of Research Grants two grant applications for conducting multicenter clinical trials, one in glaucoma and one in uveal melanoma.

Dr. Milton provides the NEI Director's Office with biostatistical, epidemiological, and administrative support through consultation, review, and collaboration for international projects in ophthalmologic research, including matters related to the International Agency for the Prevention of Blindness and to the US-Indo Science and Technology Initiative programs.

Mr. Podgor worked with Dr. Muriel Kaiser, Clinical Branch, NEI, on an extension of a study of ocular rigidity of patients with osteogenesis imperfecta. Dr. Kaiser presented this material to the Ophthalmological Society of the United Kingdom.

Mr. Podgor provided consultation to Dr. Kaiser on data collection and on the development of a computer system for data management of pigment dispersion syndrome information.

Mr. Podgor is working with Dr. Monique Roy, Clinical Branch, NEI, on conjunctival signs in patients with sickle cell disease.

Dr. Green provided consultation for National Institute of Neurological and Communicative Disorders and Stroke as member of site-visit team reviewing proposed studies of otitis media in children.

Dr. Green collaborated with Dr. David Byar, NCI, on methodology for predicting disease incidence in high-risk individuals, either for individual counseling or for planning of prevention studies.

Dr. Green consulted with Dr. Jon Currie, Clinical Branch, NEI, on analysis of pupil size in normals before and after application of a pharmacologic agent.

Dr. Green collaborated with Dr. Robert Nussenblatt of the NEI Clinical Branch on design of a randomized clinical trial comparing cyclosporine with steroid therapy for prevention of rejection of corneal transplants.

Dr. Seigel provided consultation to Dr. Nussenblatt and Dr. Alan Palestine, Clinical Branch, NEI, on randomized trials of cyclosporine.

Dr. Seigel provided consultation to Dr. Israel Goldberg of the NEI extramural program on clinical trials for eye tumors.

Dr. Seigel provided consultation to Dr. James B. Wyngaarden, NIH Director, on a contract with The National Academy of Sciences on the effectiveness of clinical trials.

Dr. Sperduto served as an ophthalmic consultant to NEI's Office of Scientific Reporting and Office of Program Planning, Analysis, and Evaluation.

Dr. Sperduto was appointed a temporary Consultant to the World Health Organization and traveled to Fiji where he assessed cataract research opportunities and prepared a report.

Dr. Sperduto provided consultation to Harvard Medical School on a clinical study of retinitis pigmentosis and allied diseases.

Mr. Ederer served as a member of the Policy and Data Monitoring Committee of the NEI Sorbinil Retinopathy Trial, and of the Policy and Data Monitoring Group for the NCI Colon Cancer Control Study.

Dr. Ferris is a member of the Data, Safety, and Quality Review Board for the Diabetes Control and Complications Trial, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. He is also a member of the DRVS Data Monitoring Committee, and Data and Safety Monitoring Committee of the grant-supported Branch Vein Occlusion Study.

Dr. Green serves on the Data Monitoring Committee of the KARNS and the Analysis Planning Committee of the ETDRS.

Dr. Mowery co-chaired a site visit team for the Lipid Metabolism-Atherogenesis Branch, NHLBI, to evaluate the progress and future plans of the coordinating center for the Lipid Research Clinics Program.

Dr. Mowery is a member of the Data Monitoring Committee for the NEI grant-supported clinical trial Retrolental Fibroplasia: Clinical and Research Aspects.

Dr. Seigel was a member of an Advisory Committee of the Bureau of Biologics, FDA, and a member of the Advisory Board of the Boston University Collaborative Drug Study.

Dr. Sperduto was chairman of a workshop on cataract at the International Congress for Tropical Medicine and Malaria. He presented a talk on "Cataract as a Cause of Blindness in the Tropics," and a second talk describing the Joint US-India Epidemiologic Study of Senile Cataract.

Dr. Sperduto was a member of the committee that reviewed the Eales' Disease Study that will be conducted under the 1983 US-Indo Science and Technology Initiative.

Dr. Sperduto is a member of the Data Monitoring Committee for a grant-supported clinical trial on retinitis pigmentosa.

Dr. Sperduto serves on an NIH committee that reviews research protocols to determine conformance to the criteria for exemption from the usual OMB survey review requirements.

Presentations

Dr. Green presented a paper entitled "Randomized Comparisons of BCNU, Streptozotocin, Radiosensitizer, and Fractionation of Radiotherapy in the Post-operative Treatment of Malignant Glioma" at the annual meeting of the American Society of Clinical Oncology.

Dr. Green presented a paper entitled "Using Case-control Data to Estimate Sample Sizes Required for Disease Prevention Trials in High-risk Groups" at the annual meeting of the Society for Clinical Trials.

Dr. Milton presented a paper at ARVO on eye disease among diabetics and non-diabetics in the Framingham Eye Study. The paper is being submitted with Peter Wilson of the National Heart, Lung, and Blood Institute, and Dr. Ferris as co-authors.

Mr. Podgor presented a paper at ARVO on associations of lens changes and survival in the Framingham Eye Study population.

Dr. Seigel gave a talk on case-control methods in nutritional research at a conference chaired by Dr. Underwood.

Dr. Seigel gave talks on competing risk theory at the annual meeting of the Society of Clinical Trials at the University of Minnesota, and at Johns Hopkins University.

Dr. Seigel lectured on clinical trials at the US-Japan Workshop on uveitis in Hawaii which was arranged by Dr. Jin Kinoshita.

Dr. Sperduto gave a talk on epidemiologic associations with cataract at the US-Europe Cataract Conference in October 1983.

Professional Activities

The National Eye Institute is represented by Mr. Ederer on the NIH Epidemiology Committee and by Dr. Seigel on the NIH Clinical Trials Committee. Dr. Green recently replaced Dr. Seigel as a representative on the NEI Institutional Review Board (IRB).

The recently formed American College of Epidemiology, through its Committee on Examination, chaired by Mr. Ederer, has prepared and administered the first examination for certifying applicants for membership in the College.

Dr. Milton is a member of the Board of Directors of the International Agency for the Prevention of Blindness.

Dr. Milton represented the International Association for Statistical Computing (IASC) on the program committee for the October 1984 Conference on Frontiers of Computational Statistics, which was jointly sponsored by the IASC, the Society for Industrial and Applied Mathematics, and the American Statistical Association.

Mr. Ederer is a member of the Editorial Board of the American Journal of Ophthalmology, and Dr. Seigel is a member of the Editorial Board of Archives of Ophthalmology. Both Mr. Ederer and Dr. Seigel are Associate Editors for the American Journal of Epidemiology.

Dr. Milton was appointed as a member of the Management Committee for the Current Index to Statistics, a joint publication of the American Statistical Association and the Institute of Mathematical Statistics.

Dr. Milton represents the NEI on the NIH Advisory Committee on Computer Usage.

Dr. Seigel was Chairman of the National Institute of Dental Research Committee on Personnel Actions in Biometry and Epidemiology.

Publications:

1. Ederer F: Methodological problems in eye disease epidemiology. Epidemiol Rev 5:51, 1983.

2. Ederer F, Podgor MJ, and the Diabetic Retinopathy Research Group: Assessing possible late treatment effects in stopping a clinical trial early: A case study. DRS Report 9. Controlled Clin Trials (in press).

3. Ederer F: Epidemiologic considerations. In Light Toxicity, Waxler M, editor. Boca Raton, Florida, CRC Press (in press).

4. Kincaid MC, Green WR, Fine SL, Ferris FL, and Patz A: An ocular clinicopathologic correlation study of six patients from the diabetic retinopathy study. Retina 3:3, 1983.

5. Ferris F and Patz A: Macular Edema: A complication of Diabetic Retinopathy. Surv Ophthalmol 20(Suppl):452, 1984.

6. Ferris F: Epidemiologic Approach to Clinical Research: Design Strategies. In Clinical Ophthalmology, Duane T, editor. Hagerstown, MD, Harper and Row, 1984, Vol. 5, Chapter 66.

7. Cassel G and Ferris F: Site visits in a multicenter ophthalmic trial. Controlled Clin Trials 5:251, 1984.

8. Murphy R and Ferris F: The current status of the Early Treatment Diabetic Retinopathy Study. Ophthalmic Forum 2:149, 1984.

9. Ferris F, Fine S, and Hyman L: Proportion of age-related macular degeneration patients blind from neovascular maculopathy. Arch Ophthalmol (in press).

10. Green SB and Byar DP: Using observational data from registries to compare treatments: The fallacy of omnimetrics. Statistics in Medicine 3 (in press).

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12. Macular Photocoagulation Study Group: Argon laser photocoagulation for ocular histoplasmosis. Arch Ophthalmol 101:1347, 1983.

13. Macular Photocoagulation Study Group: Argon laser photocoagulation for idiopathic neovascularization. Arch Ophthalmol 101:1358, 1983.

14. Newsome DA, Milton RC, and Frederique G: High prevalence of eye disease in a Haitian locale. J Trop Med Hyg 86:37, 1983.

15. Seigel D and Milton RC: Further results on a multiple-testing procedure for clinical trials. Biometrics 39:921, 1983.

16. Seigel D: Clinical Trials. In Pharmacology of the Eye, Sears ML, editor. Heidelberg, Germany, Springer-Verlag, 1984, pp. 687.

17. Seigel D: Discussion of papers by Peleg and others, and by Willett, Stahelin, and others. JNCI-Vitamin A and Cancer Prevention Conference. J Nat Cancer Inst (in press).

18. Sperduto RD, Ferris FL, Hagler WS, and Billings TE: Senile macular degeneration: An artist's view. JAMA 250:2506, 1983.

19. Sperduto RD: Cataract. In Research Priorities for the Prevention of Blindness in Developing Countries. Beyda V, editor. New York, Helen Keller International, Inc., 1984, pp. 24.

20. Sperduto RD and Hiller R: The prevalence of nuclear, cortical and posterior subcapsular lens opacities in a general population sample. Ophthalmology 91:815, 1984.

21. Visual Acuity Impairment Survey Research Group: Visual Acuity Impairment Survey Pilot Study. National Technical Information Service: PB84 156173, 1984.

CONTRACT NARRATIVE

Thirteen Clinical Centers, plus a Coordinating Center at the University of Minnesota, Minneapolis, Minnesota, and a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Principal Investigators: Matthew D. Davis, M.D. (Study Chairman)
Daniel Seigel, Sc.D. (Project Officer)

Current Fund Allocation: $212,000 for FY 1984.

Objectives: The DRVS is a multicenter clinical trial to:

1. Evaluate vitrectomy performed in the first six months after severe vitreous hemorrhage secondary to diabetic retinopathy compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous (group H).

2. Evaluate vitrectomy in eyes with good vision but with severe proliferative retinopathy and poor prognosis before vision is lost through hemorrhage or retinal detachment (group NR).

3. Study the natural history of severe proliferative diabetic retinopathy.

Major Findings: A total of 616 eyes with severe hemorrhage have been randomized to group H; 381 eyes have been randomized to group NR; and 622 patients have been recruited for the natural history study. Follow-up of patients in the natural history study has ceased, follow-up of patients in the randomized trials will continue until all patients have been observed for four years (mid-1987).

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three (macular degeneration, glaucoma, cataract) in that it generally affects a younger population. Vitrectomy has the theoretical potential of removing the "scaffolding" on which abnormal new vessels can develop, fibrous tissue can form, and retinal detachment can occur. It is important to determine when such intervention is most likely to deter this process and reduce the incidence of loss of vision.

Proposed Course: Follow-up will continue until every patient is four years past randomization (mid-1987). A manuscript on the first two years of follow-up in the natural history study has been submitted for publication. A draft of a manuscript has been prepared which features two-year follow-up in the H group. Other publications planned for the next two years are four-year follow-up in the natural history study, further analyses in the H group, and short-term results in the NR group.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities.

Publications: None

CONTRACT NARRATIVE

Twenty-three Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Fundus Photograph Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin; a Central Laboratory at the Centers for Disease Control, Atlanta, Georgia; and an Electrocardiogram Reading Center at the University of Minnesota, Minneapolis, Minnesota

Title: Early Treatment Diabetic Retinopathy Study (ETDRS)

Principal Investigators: Dr. Lloyd Aiello (Co-Chairman)
Dr. Frederick L. Ferris, III (Co-Chairman)
Dr. Richard Mowery (Project Officer)

Current Fund Allocation: $5,662,000 (estimated) for FY 1984

Objectives: The Early Treatment Diabetic Retinopathy Study (ETDRS) is a multicenter, randomized clinical trial, the main goals of which are:

1. To determine whether treatment of early stages of proliferative and nonproliferative diabetic retinopathy, with or without macular edema, by aspirin and/or prompt photocoagulation is effective in decreasing the rate of development of known retinopathy risk factors and/or the development of severe visual loss when compared to placebo or deferred photocoagulation.

2. To help determine the best time to initiate photocoagulation treatment in diabetic retinopathy.

3. To monitor closely the effects of diabetes mellitus and/or of photocoagulation on visual function.

4. To produce natural history data that can be used to develop (identify risk factors) and test etiologic hypotheses in diabetic retinopathy.

Major Findings: As of June 15, 1984, 4,386 patients had started qualifying visits for this study with 3,896 completing this visit. A total of 3,662 treatment allocations have been issued, and 3,315 patients have been treated. Recruitment will continue through March 1985, with a goal of 4,000 patients to be enrolled in the study. The study has not reported any results.

Significance to Biomedical Research and the Program of the Institute: The National Eye Institute regards fostering careful evaluation of new and widely used ophthalmic treatments as an essential element in its mission. This study represents an extension of the Institute's interest in preventing visual impairment of patients with diabetes.

Proposed Course: Follow-up of all ETDRS patients is planned to continue through December 1989. Monitoring of accumulated data is performed at quarterly intervals.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities.

Publications: None

CONTRACT NARRATIVE

Three Clinical Centers, plus a Coordinating Center at the University of Minnesota, Minneapolis, Minnesota; the Census Bureau; and a Fundus Photograph Reading Center at the Office of Biometry and Epidemiology, National Eye Institute, Bethesda, Maryland

Title: Visual Acuity Impairment Survey (VAIS) Pilot Study

Principal Investigators: Fred Ederer (Project Officer)
Richard Mowery (Deputy Project Officer)

Current Fund Allocation: $50,277 for FY 1984.

Objectives: The Visual Acuity Impairment Survey Pilot Study is a planned multicenter epidemiological study of the prevalence of central distance visual acuity impairment in the United States and of the eye diseases responsible for impairment. The main goals of the one-year study are:

1. To determine the feasibility of the VAIS.

2. To pretest home interview screening procedures and clinic examination procedures.

3. To gather information that will help to plan the full study.

Major Findings: Although many aspects of the Pilot Study demonstrated the feasibility of the VAIS plan, there was one critical difficulty, namely the small percentages of both positive and negative persons screened who were willing to come to the eye clinics for examination--52% and 54%, respectively. In addition, small percentages of persons sampled could not be located for interviews (4.5%) or refused the VAIS interview and visual acuity screening (8.4%).

The percentage participation in clinic examination was strongly and inversely related to age. For persons sampled aged 75 and older, the rates were 30% and 31% for positive and negative persons screened, while for those aged 25-74, the rates were 66% and 60%, which represents increases over the rates for all ages combined. But even these rates, because of the potential for self-selection bias, are considered to be too low by survey statisticians and by us.

Significance to Biomedical Research and the Program of the Institute: The Visual Acuity Impairment Survey originated from the Institute's past involvement with the Model Reporting Area for Blindness Statistics, the National Center for Health Statistics' Health and Nutrition Examination Survey, and the Framingham Eye Study. These studies attempted to measure the frequency of eye diseases or of visual impairment, but were limited in scope, lacked assurance of quality, or were hampered by logistical problems. The VAIS represents an extension of the Institute's interest in epidemiologic research and in gathering high quality population-based data to be used in program planning and for public information. The

Study further offers the opportunity to introduce vision researchers to epidemiologic concepts and methods.

Proposed Course: The staff of the Office of Biometry and Epidemiology prepared a detailed report on the VAIS pilot study in January 1984. The staff is presently working with the investigators to prepare five manuscripts from the data collected. Collaborating with staff from the National Center for Health Statistics, the Office of Biometry and Epidemiology staff is exploring the possibility of a joint collaboration on the 1987 Health and Nutrition Examination Survey, which was the major recommendation from the VAIS pilot study report.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities.

Publications:

1. Visual Acuity Impairment Survey Research Group: Visual Acuity Impairment Survey Pilot Study. National Technical Information Service: PB34 156173, 1984.

OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION

Julian M. Morris

Planning and Evaluation Section

During FY 1984, the Office completed publication and distribution of Vision Research--A National Plan: 1983-1987. We also continued refining our system for tracking grant applications and funded projects in terms of their relevance to the recommendations of this National Plan, began preparations for its "mid-course" evaluation, and prepared a timetable for the development of the 1988-1992 Plan.

Office staff engaged in a variety of other activities during the year, the most significant of which are highlighted below. These include reports the staff drafted, contributed to, commented upon, or coordinated in response to requests from NIH, PHS, the Department, or outside organizations.

- Organization of NEI participation in the Annual Planning Session with the NIH Director in preparation for the 1985 Appropriations Hearings and subsequent drafting of the NEI contribution to the NIH Research Plan for FY 1985-1988 based on the outcome of this session.
- "Report to the House Appropriations Committee on Implementation of the First Year Objectives of the NEI Five-Year Plan."
- Information on sight restoration procedures to the Congressional Subcommittee on Public Assistance and Development Compensation.
- Congressional Appropriations Committee Report on Multiple Sclerosis.
- "FY 1984 DHHS Toxicology Plan."
- "1984 NCI Director's Report and Annual Plan."
- NIH Annual Immunology Report to the Congress.
- Support of Trauma and Burn Research, FY 1983.
- Estimates for Nutrition-Related Research in FY 1982-1984.
- Submission for NIH Nutrition Priorities.
- Support in FY 1983 for research related to the Maternal and Child Health Inventory.
- Biotechnology report for the Secretary.
- Information for the Secretary's statement to Congress on Health Information and Promotion.

- Support for Rare Diseases, FY 1983.

- Annual Report on the Decade of Disabled Persons.

- NEI position on the American Academy of Ophthalmology's National Eye Care Project.

- NEI position on the draft report of the Committee on the NIH Evaluation Program.

- Description of the implementations and uses of three NEI program Evaluation projects.

- Background information on NEI support for neurobiology, molecular biology, and immunology for planning meeting between the Assistant Secretary for Health and the NIH Director.

- Prevention 84, the Biennial Report on DHHS prevention activities.

- Prevention-Related Research Report, FY 1983.

- Prevention Health Screening and Detection Program Report.

- Summary of NEI activities in AIDS research.

- Response to National Initiative for Glaucoma Control.

- NEI additional needs and opportunities in research and research training related to biotechnology and the broader science base underlying biotechnology.

- NIH report to Senator D'Amato on examples of cost-effective NIH-supported research.

- NEI support for biomaterials in FY 1983-1985.

During the past year Mr. Morris:

- Served as NEI liaison for the Institute of Medicine (IOM) Study of the Organizational Structure of the NIH; in particular, he worked closely with the IOM subcontractor to assure the accuracy and comprehensiveness of the NEI Case History, which was developed as a background document for the Study.

- Supervised the maintenance and further development of the NEI's I2I Management Information System which included the consolidation or elimination of several recurring reports, the enhancement of others, and the development of a report that for the first time gives the NEI management a detailed and accurate account in one data presentation of extramural expenditures to date, future commitments, and unexpended balances remaining.

- Assumed responsibilities as Project Officer for NEI's annual professional educational course on Clinical Vision Research.

- o Advised the NEI Director on program evaluation needs for the International Vitamin A Consultative Group.

- o Chaired the NEI's Office Automation Task Force and spearheaded an effort that led to the selection of and planning for a new NEI-wide office automation system to be implemented in several phases beginning in FY 1985.

- o Served as Project Officer for a study of the NEI word processing capabilities and needs conducted by the General Services Administration.

Mr. Morris also continued in his roles as NEI Prevention Coordinator and NEI Legislative Liaison, and he served on the NEI Institute Review Board for evaluating proposed NEI Clinical Branch research protocols.

Among his duties and responsibilities during the past year, Mr. Gillen wrote an article on blindness for Collier's Encyclopedia, served as the NEI representative to the NIH Task Force on Nursing Research, and, as in past years, supervised coordination of the NEI Annual Report.

During FY 1984, Michael Davis joined the Office as a Program Analyst. Mr. Davis previously worked for the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, where he was a Health Systems Specialist with the Phoenix Clinical Research Section in Arizona. He has training in microbiology and administration and will have major responsibility for NEI's participation in Trans-NIH analyses and reporting, for the scientific coding of NEI's extramural and intramural projects, and for tracking the implementation of our five-year Plan.

Program Analysis Section

During FY 1984, the Program Analysis Section (PAS) conducted an in-depth analysis of the National Eye Institute's fiscal recording and reporting procedures. Among other things, this evaluation led to significant streamlining and automation of the duties that had been performed by the Section's fiscal analyst, who left the NEI, allowing PAS to continue these functions with a greater degree of accuracy yet without needing to refill that position.

A pivotal element in this process was the development of I2I report #1095 by staff programmer Carolyn McIntyre in conjunction with NEI consultant Gaye Lynch. This report provides a weekly up-to-date accounting of expenditures and commitments for all NEI research grants. The report and variants of it have superseded paper records that had been maintained by both the Extramural Services Branch of the Extramural and Collaborative Programs and PAS and have also provided more accurate and up-to-date information to the NEI Budget Office. This report and the coordinated learning process that transpired among the PAS, grants management, and budget office staffs in developing it have greatly enhanced the cooperation and synchronization among these three offices in the fiscal accounting of grants.

PAS has also continued to streamline and improve other management information services to the NEI. Last year, its staff wrote over five hundred new custom programs in response to requests from other Institute staff, most of which were filled within twenty-four hours. This number does not include reruns or minor adaptations from PAS's total catalogued base of fourteen hundred programs, which satisfies a large portion of its information requests. In addition, PAS has made further improvements in its standard periodic runs (weekly, monthly, quarterly, pre- and post-Council, and annual) and over the past year completed the conversion of these runs from IRS, an outdated, cumbersome programming language, to its new I2I system. PAS has also continued its function of maintaining and expanding as needed the NEI's grants, contracts, trainee, and intramural data bases.

Other efforts performed by PAS during the past year include the following:

- Development of a series of statistical and graphical procedures to monitor grant activity within the Plan's program planning tracking categories as a basis for updating the Plan.

- Establishment of two databases and preparation of associated reports of international programs for the prevention of blindness and for international support for vision research.

- Procurement and installation of computer hardware and software to produce within the Office color graphic displays of program information for the use of OPPAE and other NEI staff offices.

- Expansion of search services to NEI users from the MEDLARS data bases, including MEDLINE, TOXLINE, and CANCERLINE.

- Installation of new quality control procedures to assure accuracy of program coding.

- Leadership in the development of plans for an office automation information exchange for NIH's Office Technology Coordinating Committee.

- Development of a computer logging system to track new Small Grants (R03) and new data entry procedures for Small Business Grants (R43).

OFFICE OF SCIENTIFIC REPORTING

ANNUAL REPORT
NATIONAL EYE INSTITUTE
OCTOBER 1, 1983 - SEPTEMBER 30, 1984

REPORT OF THE CHIEF, OFFICE OF SCIENTIFIC REPORTING

Marsha S. Corbett

The National Eye Institute (NEI) is responsible both for supporting and conducting research on eye disease and vision. During Fiscal Year 1984, research conducted by NEI intramural scientists and clinicians became the focal point for many activities within the Office of Scientific Reporting (OSR). Efforts made in this regard are described below.

Intramural Research Publicity

An intensive media relations and education program was launched to publicize studies conducted by the NEI's Clinical Branch. The primary objective of this program was to assist in recruiting patients for several new clinical trials and, secondarily, to increase awareness of the NEI's clinical research program among Washington area health care professionals.

One of the new research projects is part of a nationwide clinical trial involving 12 eye care centers. In this Sorbinil Retinopathy Trial (SRT), physicians will be testing a new investigational drug called sorbinil to find out if it can prevent or slow down the eye and nerve damage caused by diabetes. To increase patient enrollment, OSR employed traditional as well as innovative methods of disseminating news of the study and the need for patients. Staff members prepared written materials, wrote an article on the study for the NIH Record, and sent announcements which were published in: eye journals and related publications; diabetes publications and newsletters; newspapers in the District of Columbia, Maryland, and Virginia; college and university newspapers in these areas; and house organs for hospitals and medical centers which serve diabetics. Paid advertisements and public service messages appeared in the Washington Post, Washingtonian Magazine, and in suburban papers as well. OSR also was successful in placing information about the study in nationally syndicated newspaper articles and columns. And, it spearheaded efforts to give SRT information to physicians with diabetic patients who participated in workshops sponsored by the American Diabetes Association.

In addition to the above, the Office employed innovative methods to identify and disseminate information to good prospects for the study--people who have been taking insulin for 1 to 15 years. Posters were designed and printed for point-of-purchase display in pharmacies and supermarkets throughout the metropolitan area. OSR negotiated with several chain stores, which agreed to distribute the posters, at no cost to the NEI, to prescription drug counters where diabetics buy their medication. They were also placed in public libraries throughout Northern Virginia, Maryland, and the District of Columbia, and eventually will be posted in hospital-based diabetes clinics. To accompany the posters, OSR produced pamphlets for pharmacists to give to diabetics having insulin prescriptions filled. SRT information was also sent to diabetologists and included in testimony prepared for Senate and House Appropriations Hearings and in the Congressional Record.

The same sort of activities were undertaken on behalf of another study being conducted by Clinical Branch investigators. They are seeking patients who have early signs of senile macular degeneration to determine whether a combination of medication and protective sunglasses can prevent progression of this eye disease or decrease its severity. OSR sent announcements and articles to: newspapers in the Washington area; ophthalmology, optometry, eye research, and aging-related publications; the NIH Record; newsletters produced by associations of retired teachers, nurses, and golden age groups; hospitals and medical centers with aging-related programs; senior citizen centers; homes for the aged; nursing homes; and Health Maintenance Organizations. In addition, advertisements and articles appeared in daily and weekly newspapers.

The Office incorporated information about other intramural studies and basic and clinical research advances into newspaper articles and columns, Opening Statements at Senate and Congressional Appropriations Hearings; Special Reports to Congress; an NEI exhibit to be displayed in the Office of the Secretary, HHS; fact sheets; and copy supplied to newspaper, television, and radio reporters.

Extramural Research Publicity

The OSR also provided media relations, public education, scientific reporting, and knowledge transfer services in support of the Institute's Extramural and Collaborative Programs.

The Office developed and implemented an information dissemination plan for reporting new research results on laser treatment of presumed ocular histoplasmosis. Written materials were prepared for distribution, and the plan was carried out by the 22 medical centers participating in the study.

Information about this and other multicenter clinical trials supported by the NEI was also communicated in exhibits for health care professionals, scientists, and laymen; health fairs; museums; libraries; schools; Presidential proclamations drafted by OSR and other components of NIH; inter-agency reports; Opening Statements at Senate and Congressional Appropriations Hearings; Special Reports to Congress; fact sheets and brochures; journals and other medical and health publications; and nationally syndicated newspaper articles.

In addition to the above, the Office developed and refined a new approach to the dissemination of clinically applicable research results: organizations in the private sector are now reprinting and distributing OSR brochures at no expense to the NEI. OSR supplies negatives of these brochures which the cooperating organizations are welcome to print with their own name or logo. In this way, OSR descriptions of research results are being communicated to a far larger audience than the NEI could hope to reach with its own limited resources.

This innovative approach to disseminating information about eye disorders and advances in vision research has been applied primarily to the communication of sight-saving results from the national collaborative Diabetic Retinopathy Study supported by the NEI. Of course OSR also continues to employ other techniques to disseminate these results, in cooperation with organizations and agencies that distribute printed materials directly to diabetics and their families. Organizations with which OSR has collaborated in this way include the following: Juvenile Diabetes Foundation; American Diabetes Association; American Association of Diabetes Educators; Project Orbis; Vision Foundation; Information

for the Partially Sighted; National Diabetes Information Clearinghouse; National Society to Prevent Blindness; American Academy of Ophthalmology; American Optometric Association; Lions Clubs International; Centers for Disease Control's Diabetes Control Program; and organizations representing nurses, pharmacists, hospitals, and public libraries.

The OSR also has continued to work with the International Agency for the Prevention of Blindness (IAPB) and other international organizations committed to the prevention of avoidable blindness and visual impairment in developing countries. OSR staff designed an IAPB exhibit for display at the American Academy of Ophthalmology meeting in November, 1983, and prepared fact sheets and art-quality posters for distribution. The NEI's involvement with international blindness prevention activities, combined with its own US-India collaborative research projects, have generated an increasing number of inquiries and requests for information on these and related subjects.

Other Activities and Functions

In addition to the projects described above, which were a primary focus of activity within OSR during the past fiscal year, the office continued to bear responsibility for the following routine information office functions: scientific reporting and knowledge transfer, consumer education, media relations, public inquiries, and Freedom of Information Act requests.

In carrying out these functions, OSR provided information on a vast array of subjects to: ophthalmologists; optometrists; epidemiologists; neuroscientists; nurses; diabetes educators; voluntary associations; professional societies; philanthropic organizations; Senate and House committees; other components of NIH and the Federal Government; state and local governmental bodies; reporters for radio, television, and the print media; U.S. citizens; and people from other countries. At times during the past year, requests for information and for printed materials from these individuals and groups increased a hundredfold over OSR's normal volume. Thanks to continued streamlining of OSR's inquiry and order filling system, the availability of a few new fact sheets and a new booklet on cataract, and the timely assistance of other components of the NEI, these requests were handled promptly.

INTRAMURAL RESEARCH

ANNUAL REPORT
National Eye Institute
October 1, 1983 - September 30, 1984

REPORT OF THE SCIENTIFIC DIRECTOR

Jin H. Kinoshita, Ph.D.

During the past year there have been a number of major changes in personnel and organizational matters in the NEI intramural program. The new development in the organization of our program was the establishment of the Laboratory of Pathology with Dr. Toichiro Kuwabara as its Chief. Dr. Kuwabara, a renowned ophthalmic pathologist, was previously Head of the Pathology Section of the Laboratory of Vision Research. Dr. David G. Cogan of the Clinical Branch will serve as Deputy Chief of the Laboratory. This brings together in a single organization the team of Cogan and Kuwabara which for the past three decades has had a profound impact on the field with its numerous provocative and innovative research in matters related to diabetic retinopathy, cystinosis, aberrant lipogenesis of the cornea, histochemistry of the eye, cataracts, and light damage effects. For these and related studies this team has received numerous awards from the Association for Research in Vision and Ophthalmology, American Medical Association, American Ophthalmological Society, and Research to Prevent Blindness, Inc. Thus, the new Laboratory of Pathology initiates its research program with an illustrious and distinguished leadership. In addition, Dr. Merlyn Rodrigues, another senior and established eye pathologist who was a section head in the Clinical Branch, has also joined the Laboratory. The nucleus of the Laboratory is formed by these senior investigators along with two promising young scientists interested in pathology, Dr. Gerald Robison and Dr. George Inana, who have transferred from the LVR and LMDB. Thus, this Laboratory is composed of experienced and young scientists who should provide leadership to the field of ophthalmic pathology for years to come.

A major development in the NEI Clinical Branch was the selection of Dr. Robert Nussenblatt as Deputy Clinical Director. This brings into a position of leadership a highly successful clinical researcher. Dr. Nussenblatt will have to balance the demands of new adminstrative duties with those of expanding his highly acclaimed research program on inflammatory disease processes. The decision of this appointment was necessary to fill the void caused by Dr. Elmer Ballintine's retirement as Clinical Director. Dr. Nussenblatt will be responsible for the day-to-day operations of the Clinical Branch activities. He shares the responsibility of running the Branch with Dr. Carl Kupfer who will continue as Acting Clinical Director.

Dr. Douglas Gaasterland who has been at the NEI for the past 14 years and was Head of the Clinical Branch's Glaucoma Section, has assumed a new position at Georgetown University Medical School. Dr. Gaasterland's departure is a serious loss and leaves another void in the Clinical Branch. This represents a disturbing trend. We are having difficulty in retaining our accomplished ophthalmic scientists because we cannot compete favorably with offers made to them by medical schools.

During the past year there was a change in the important position of Administrative Officer of the intramural program. Mr. C. L. Napper was the Administrative Officer since the inception of the NEI intramural program. He played an important role in organizing and establishing the various branches of the intramural program. We who have witnessed the growth and development of the intramural program deeply appreciate his contribution to the success of the program. Mr. Napper leaves the intramural program for another important position in our institute. Ms. Karen Wright is currently serving as the Acting Administrative Officer.

Despite these changes and the disruptions caused by moves into newly renovated laboratory and clinical space which are still continuing, we are impressed by the research accomplishments of the scientists. Currently we have to contend with serious constraints in the budget and the necessity of reducing our research staff. We are encouraged, however, that long range plans of stressing key research programs are beginning to show results. These facts can be gleaned by perusing the information contained in this year's Annual Report.

CLINICAL BRANCH

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE DEPUTY CLINICAL DIRECTOR

Robert B. Nussenblatt, M.D.

The Clinical Branch of the NEI has seen its clinical and research program evolve and grow over the past year. The year saw essentially all of our clinical services moved to the 10th floor of the Clinical Center and the ACRF. This move occurred with no disruption of patient services, in spite of the ever-increasing inpatient and outpatient responsibilities of the Clinical Branch, in part due to three large scale double-masked randomized studies. The Branch is composed of five sections, each with its own section head: Section on Clinical Ophthalmic Immunology, Robert B. Nussenblatt, M.D.; Section on Glaucoma, Douglas E. Gaasterland, M.D.; Section on Neuro-ophthalmology, David G. Cogan, M.D.; Section on Ophthalmic Genetics and Pediatric Ophthalmology, Muriel I. Kaiser-Kupfer, M.D.; and Section on Visual Processing, Francisco deMonasterio, M.D., D.Sc. Forty clinical research projects were active during fiscal year 1984.

The Section on Clinical Ophthalmic Immunology investigates ocular disorders of immune mediation using a variety of methods. The group has continued to evaluate the underlying mechanisms of S-antigen-induced uveitis in lower mammals. An inducer T-cell line has been identified that is capable of producing this disorder when transferred to naive rats. It was shown as well, using laser cytofluorograph analysis of T-cell populations from various organs, that cyclosporine appears to affect the inducer cell fraction, with this perturbation important in that agent's effective abrogation of the disease. The first double-masked randomized study in uveitis has begun to compare the effectiveness of cyclosporine therapy with that of systemic corticosteroids. The Section has also begun to investigate the role of the immune system in several ocular diseases. The finding that patients with retinitis pigmentosa produce low amounts of gamma interferon, and have an altered expression of cell surface antigens, and that a subgroup have in vitro responses to retinal antigens has opened a new area of investigation for this disorder for which, until now, has had no known therapy.

Investigators in the Section on Glaucoma have continued their studies evaluating the mechanisms of action and role in ophthalmology of lasers. A systematic evaluation of the effects of laser in simian eyes continued. Patients with anterior segment alterations have been treated with either mode-locked or Q-switched neodymium-YAG lasers. One hundred and one patients have completed a followup from six months to one year. A greater than 95% efficacy in terms of achieving the goals of treatment appears to have been achieved. The evaluation of these results will provide important information to those involved in this ever changing field.

The Neuro-ophthalmology Section has had a long-standing interest in oculomotor disorders, with detailed evaluation centering around such entities as congenital nystagmus, Parinaud's syndrome, and ocular motor palsies. Other endeavors have included studies of the incipient stages of Alzheimer's disease,

which can present as a diagnostic dilemma because of its focal visual symptomotology. The videotape and oculographic recordings provide an extensive, invaluable library in the field of neuro-ophthalmology.

Scientists in the Section on Ophthalmic Genetics and Pediatric Ophthalmology have continued to recruit and evaluate patients with gyrate atrophy, an autosomal recessive disorder associated with hyperornithemia. A low arginine diet has been initiated in many of these patients as a form of therapeutic intervention. In patients with good biochemical control, initial indications suggest that this approach may provide very promising results. Additionally, the Clinical Branch is participating in a multi-center, double-masked, randomized study evaluating the effects of the aldose reductase inhibitor, sorbinil on diabetic retinopathy. Scientists in this section actively participate in the NIH Interinstitute Medical Genetics Program. Because of the high frequency of ocular involvement in many of the cases, almost all the patients were evaluated by Clinical Branch staff or were discussed in consultation.

The Section on Visual Processing provides the supportive electrophysiologic and psychophysical testing for both inpatients and outpatients, as well as those patients seen in consultation. In addition to this supportive role, the Section has maintained active research activities. In the clinical realm, these endeavors have focussed on the forced choice spatial contrast sensitivity and visual evoked responses. These newer techniques provide more precise mechanisms by which alterations in ocular disease can be measured. The Section staff continues their basic anatomical and physiological studies which center about single cell properties in the retinae of nonhuman primates.

The Clinical Branch continues to be at the cutting edge of multiple areas of ophthalmic research. The following reports will provide the reader a more detailed description of these activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00143-11 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Douglas E. Gaasterland M.D. Head, Section on Glaucoma CB, NEI

Others: Elmer J. Ballintine M.D. Senior Clinical Advisor CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Branch

SECTION

Section on Glaucoma

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thirty-six patients with pigmented ocular lesions originally joined this study (1972-1973). The early results of the study demonstrated that the diagnostic technique had inadequate specificity. For most patients a clear clinical diagnosis has been made, and their ocular problem solved. Occasional follow-up examinations have continued during this year, but the project if now completed.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None.

Protocol Number: 76-EI-370

Objectives: To determine the value of using I-125 labeled chloroquine analog for the detection of ocular melanoma.

Methods Employed: Follow-up clinical examinations are performed.

Major Findings: Of the five patients being followed chronologically in the study one was lost to follow-up during the year due to severe, prolonged debilitating cardiovascular disease. The other patients were seen with an essentially stable condition. They have now been discharged from follow-up. No new observations were made.

Significance to Biomedical Research and the Program of the Institute: Continued follow-up of the patients is important because it gives information about the course of the disease with and without treatment.

Proposed Course: The project is terminated.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00168-09 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laser Surgery for Glaucoma and Other Anterior Segment Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Douglas E. Gaasterland	M.D.	Head, Section on Glaucoma	CB, NEI
Others:	Charles Bonney	D.V.M., Ph.D.	Visiting Scientist	CB, NEI
	Elmer J. Ballintine	M.D.	Senior Clinical Advisor	CB, NEI
	Claude E. Cummins, III	B.S.	Medical Student, Biologist	CB, NEI

COOPERATING UNITS *(if any)*
Biomedical Engineering and Instrumentation Branch, DRS (R. Bonner, Alan Rich, T. Tedder); University of Texas Health Sciences Center, San Antonio; NHLBI.

LAB/BRANCH
Clinical Branch

SECTION
Section on Glaucoma

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.6	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The high energy and power of lasers offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, iridotomy and trabeculotomy are possible. This has importance for glaucoma patients because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in simian (rhesus) eyes and the application of promising systems and procedures to human glaucoma eyes under controlled conditions. Patients with anterior segment problems justifying pulsed laser intervention have been treated with mode-locked and Q-switched neodymium-YAG lasers to perform discission in some, and irridectomy in others.

Project Description:

Other Professional Personnel Engaged on Project:

Sumana K. Davi	Ph.D.	Expert	CB, NEI
Merlyn Rodrigues	M.D., Ph.D.	Head, Section on Clinical Eye Pathology	CB, NEI
Gunter Thomas	M.S.(eq)	Microbiologist	CB, NEI
Richard Weiblinger	B.S.	Biologist	CB, NEI

Protocol Numbers: 80-EI-91, 81-EI-204, 82-EI-197, 83-EI-61

Objectives: To develop workable laser systems for anterior segment surgery and to test these systems in the normal monkey eye. To study the physiologic and morphologic effects of laser energy upon monkey eyes. To apply favorable laser systems under controlled conditions to the treatment of the glaucomas and other anterior segment problems in humans.

Methods Employed: Instruments are being developed to meet the unique requirements of ophthalmic application. Standard laboratory physiologic and histopathologic (including light microscopy, SEM, and TEM) techniques are employed to study laser effects. The CB's laser laboratory is equipped with a modified model 800 Coherent Radiation argon laser, which has been used for this and other projects, three short pulse (high power) neodymium-YAG lasers, and delivery systems.

Major Findings: During FY 1984, studies of efficacy and safety of the pulsed laser systems for discission of hazy posterior capsules were completed. One hundred and one patients completed follow-up ranging from 6 months to one year. These studies demonstrated greater than 95% efficacy in terms of achieving the goals of treatment. Improvements of visual acuity range from from remarkable (9/200 to 20/15 in some cases) to none at all. After an initial improvement, two patients suffered a subsequent loss of sharpness of vision back to the pretreatment level because of cystoid macular edema. In one, this was temporary and cleared by six months. Two major complications were encountered in this study. First, many treated eyes experienced a brief spike of intraocular pressure. This resolved spontaneously in all patients without previous history of glaucoma or ocular hypertension. In two patients with previous history of this problem, the resolution was slow, taking months. Second, particularly early in the study, with a posterior chamber intraocular lens and a hazy posterior capsule against it, laser marks were created on the intraocular lens. These small pits proved to be permanent, but caused no discernible problem for the eye.

In a study that detailed the intraocular pressure response to pulsed laser discission, thirty-one patients underwent Q-switched neodymium-YAG laser discission in one eye. One-third of the patients had a pressure spike above 30 millimeters of mercury. These patients had normal aqueous normal aqueous dynamics prior to treatment. Four of the patients had pressures exceeding 40 millimeters of mercury, three of whom required medical intervention. In all patients pressure returned to normal in 24 hours and was normal at one week post-treatment without medications. This study confirmed the improvement of visual acuity in the majority of treated patients. Four additional patients entered the study of laser treatment for open angle glaucoma. Three were randomized to argon laser treatment, and one to neodymium-YAG laser treatment. One of the patients receiving argon laser treatment and the one patient receiving neodymium-YAG laser treatment did poorly, and required additional intervention. Results of this study are inconclusive.

Additional development of the frequency-doubled neodymium-YAG laser occurred during the year, allowing initial physical testing. The delivered spot size from this laser is approximately 20 micra with a maximum energy of 8 millijoules per pulse at the target site. Penetration of inanimate targets by this laser is impressive, and exceeds the penetration observed after similar treatment of the same target with the primary frequency, commercial, neodymium-YAG laser systems.

Significance to Biomedical Research and the Program of the Institute: Conceivably, a physically noninvasive laser system for anterior segment surgery could replace conventional invasive operative procedures for treating some types of glaucoma and other anterior segment problems.

Proposed Course: The project has been discontinued.

NEI Research Program: Glaucoma--Other Glaucomas (Angle-Closure Glaucoma)

Publications:

Gaasterland DE and Bonney C: Longer term effects of Q-switched ruby laser on monkey trabecular tissue. Invest Ophthalmol Vis Sci (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00046-08 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Laboratory Studies of Aqueous Humor Dynamics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Douglas E. Gaasterland	M.D.	Head, Section on Glaucoma	CB, NEI
Others:	Claude E. Cummins, III	B.S.	Medical Student, Biologist	CB, NEI
	Elmer J. Ballintine	M.D.	Senior Clinical Advisor	CB, NEI
	Richard P. Weiblinger	B.S.	Biologist	CB, NEI

COOPERATING UNITS *(if any)*
Mineralized Tissue Research Branch, NIDR (P. G. Robey, J. Kirshner).

LAB/BRANCH
Clinical Branch

SECTION
Section on Glaucoma

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.06	0.01	0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations have been done to clarify intraocular fluid movement in rhesus monkeys and humans. Monkey aqueous humor has been analyzed for glycosaminoglycans, glycoprotein, and hyaluronidase content. Human and monkey trabecular meshwork organ culture has allowed us to start defining normal and glaucomatous tissue proteoglycans production and to define the effect of glaucoma treatment on outflow tissue production of proteoglycans.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None.

Objectives: The goal of this project is to clarify aspects of intraocular fluid movement and resistance to aqueous humor outflow from the eye.

Methods Employed: Standard methods of ocular chamber and microvascular cannulation, perfusion, and noninvasive and invasive pressure measurements, with determination of volumes and flow by weight changes, dilution, or turnover techniques have been used. Aqueous obtained by ocular cannulation is analyzed for concentrations of constituents. Trabecular tissue from freshly enucleated eyes or trabeculectomy is maintained in organ culture, and the incorporation of labelled precursors of extracellular matrix components is quantitated.

Major Findings: Additional studies have been carried out on organ cultured trabecular tissue demonstrating inconclusively inhibition of uptake of precursors of proteoglycans by several glaucoma treatment medications in the media.

Significance to Biomedical Research and the Program of the Institute: The studies are elucidating the normal dynamics of aqueous humor, as well as abnormal dynamics in experimentally induced situations, mimicking clinical problems. These studies are yielding information applicable to understanding the causes and treatment of glaucoma and hypotony.

Proposed Course: The study will be discontinued.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma (Aqueous Humor Dynamics: Outflow)

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00077-07 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Treatment of Neovascular Glaucoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Douglas E. Gaasterland	MD	Head, Section on Glaucoma	CB, NEI
Others:	Elmer J. Ballintine	MD	Senior Clinical Advisor	CB, NEI
	Claude E. Cummins, III		Medical Student, Biologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Glaucoma

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There has been no new activity in this project during this year and the project is being terminated.

Project Description:

Other Professional Personnel Engaged on Project: None.

Protocol Number: 78-EI-17

Objectives: To determine whether one of two methods for ciliary body ablation, cyclodiathermy or cyclocryotherapy, is better for treatment of neovascular glaucoma.

Methods Employed: Patients who are eligible to join the study, and who consent to participate, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and identifying complications.

Major Findings: No new patients entered the study during FY 1984.

Significance to Biomedical Research and the Program of the Institute: This study has potential for indicating the proper management of these difficult secondary glaucoma patients.

Proposed Course: This study is being discontinued.

NEI Research Program: Glaucoma--Other Glaucomas (Secondary Glaucomas)

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00086-06 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Contributions to Ophthalmic Pathology and Systemic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G.Cogan	M.D.	Head, Section on Neuro-Ophthalmology	CB, NEI
Others:	Toichiro Kuwabara	M.D.	Chief, Laboratory of Ophthalmic Pathology	LOP, NEI
	Fred C Chu	M.D.	Senior Staff Fellow	CB, NEI
	Merlyn Rodrigues	M.D.	Head, Section on Ophthalmic Pathology	CB, NEI

COOPERATING UNITS *(if any)* Eunice Kennedy Shriver Center for Mental Retardation, Waltham, MA (E. Kolodny); Endocrinology Division, New England Medical Center, Boston, MA (E. Schaefer); Brigham and Women's Hospital, Boston, MA (S. Driscoll); University of Pennsylvania School of Medicine (W. Clark and D. Elder).

LAB/BRANCH
Clinical Branch

SECTION
Section on Neuro-Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.6	.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ocular abnormalities have been characterized in the eyes of patients with the following conditions: gangliosidoses of the fetus, idiopathic midline destructive disease, Gaucher's disease, abetalipoproteinemia, hereditary cutaneous melanoma and the dysplastic nevus syndrome. An additional study was a comparison of retinal and cerebro-cortical capillaries.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

John Barranger	M.D.	Deputy Chief	IRP, NINCDS
Anthony Fauci	M.D.	Chief	LIR, NIAID
Mark H. Greene	M.D.	Deputy Chief	EEB, NCI
Reginald Sanders	B.A.	COSTEP Student	EEB, NCI

Objectives: Study of clinicopathologic processes by means of histology and electron microscopy

Methods Employed: Routine processing of tissue.

Major Findings: Studies which were sufficiently complete to be reported in the literature this past year were:

1. Demonstration of abnormal cytoplasmic inclusions in retinal ganglion cells of 21-week-old fetuses with Tay-Sachs disease and with generalized gangliosidosis.

2. Necrobiotic granulomas of the lids were documented in a patient with the entity known as idiopathic midline destructive disease.

3. The alleged presence of Gaucher cells in the pingueculae of patients with Gaucher's disease was not supported in biopsy specimen from ten patients with this disease.

4. Comparison of retinal capillaries with those of the cerebral cortex showed minor differences. The latter had somewhat larger lumina, more abundant collateral channels and less regularly placed intramural pericytes.

5. A survey of 26 patients with hereditary cutaneous melanoma or the dysplastic nevus syndrome did not provide convincing evidence for significant association with intraocular nevi or melanomas.

6. The subject of damage to the eye by photic irradiation was introduced for a FDA workshop by a review of the lesions caused by various portions of the electromagnetic spectrum and its corpusclar counterparts.

7. The ocular histopathology and electron microscopy of abetalipoproteinemia were correlated with the clinical findings in a patient who was followed for many years in the NEI before finally succumbing to an unrelated tumor of the central nervous system.

Significance to Biomedical Research and the Program of the Institute: Pathology underlies disease. To understand malfunction of the body it is essential to study tissue changes wherever and whenever the opportunity arises. It is believed that the described studies represent largely original observations.

Proposed Course: Continuation of miscellaneous studies as opportunities arise.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Cogan DG, Kuwabara T, Kolodny E, and Driscoll S: Gangliosidoses and the fetal retina. Ophthalmology 91:508, 1984.

Chu FC, Rodrigues MM, Cogan DG, and Fauci AS: The pathology of idiopathic midline destructive disease (IMDD) in the eyelid. Ophthalmology 90:1385, 1983.

Chu FC, Rodrigues MM, Cogan DG, and Barranger JA: The pathology of pingueculae in Gaucher's disease. Ophthalmic Paediatrics and Genetics 4(1):7, 1984.

Cogan DG and Kuwabara T: Comparison of retinal and cerebral vasculature in Trypsin digest preparations. Br J Ophthalmol 68(1):10, 1984.

Greene MH, Sanders RJ, Chu FC, Clark WH Jr, Elder DE, and Cogan DG: The familial occurrence of cutaneous melanoma, intraocular melanoma, and the dysplastic nevus syndrome. Am J Ophthalmol 96:238, 1983.

Cogan DG: Visual health and optical radiation. In Historical Perspectives, Waxler M and Hitchins V, editors. Boca Raton, CRC Press Inc (in press).

Cogan DG, Chu FC, Rodrigues M, and Schaeffer E: Ocular abnormalities in abetalipoproteinemia. A clinicopathologic correlation. Ophthalmology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00089-06 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Eye and Metabolic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Cogan	M.D.	Head, Section on Neuro-ophthalmology	CB, NEI
Others:	Fred C. Chu	M.D.	Senior Staff Fellow	CB, NEI
	Manual Datiles	M.D.	Visiting Scientist	CB, NEI
	Peter Kador	Ph.D.	Research Chemist	LVR, NEI
	Jin Kinoshita	Ph.D.	Scientific Director	NEI
	Carl Kupfer	M.D.	Director	NEI
	Gerald Robison	Ph.D.	Geneticist/Cell Biologist	LVR, NEI

COOPERATING UNITS *(if any)*

Department of Ophthalmology, Duke University (M. Cobo).

LAB/BRANCH

Clinical Branch

SECTION

Section on Neuro-ophthalmology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.3	.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space providad.)*

An NIH Symposium on aldose reductase emphasized its practical and therapeutic implications. A note on gangliosidosis AB documented the reports of the index case and reviewed the current notion of its pathogenesis. Two cases of the Macula Halo Syndrome confirmed the one previous report of a sphingomyelinase deficiency in this entity. An update of the supranuclear motor system in metabolic disease included additional patients with Gaucher's disease and with the Niemann-Pick variant (DAF syndrome).

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

John Barranger	M.D.	Chief	DMB, NINCDS
Richard E. Gregg	M.D.	Medical Staff Fellow	MDB, NIHLB

Objectives: To identify and characterize abnormalities in metabolic disease with special emphasis on those affecting the nervous system.

Methods Employed: Appropriate patient referrals by ophthalmic and neurologic colleagues are screened for visual and oculomotor abnormalities. Those who were found suitable for further studies were, subject to their consent, enrolled in a battery of appropriate tests. Ancillary tests were enzyme determinations and conjunctival biopsies when indicated.

Major findings:

1. A departure from the customary methodology of this project has been an update on aldose reductase. Although the symposium was organized under the framework of this Section, it dealt primarily with the long-time observation of Dr. Kinoshita and his colleagues emphasizing the practical and possibly therapeutic implications for diabetes.

2. Another departure from the customary methodology was a note on the origin and present status of that Tay-Sachs variant known as gangliosidosis AB.

3. The entity which we have called macula halo syndrome has now been established as a variant of Niemann-Pick's disease. Two patients are described showing deficiency of sphingomyelinase confirming the observation on one case previously reported.

4. A review of patients with supranuclear motor symptoms in metabolic disease included the following studies:

In a cohort population of more than 100 patients with Gaucher's disease we found 18 with a significant ocular motor abnormality. Four patients with the infantile neuropathic form of the disease had total ophthalmoplegia and esotropia. Fourteen patients with the childhood neuropathic form had a characteristic abnormality of horizontal saccades, with intact pursuit movements, simulating congenital ocular motor apraxia.

Two cases have now been added to our series of nine patients with the Niemann-Pick variant (DAF syndrome), confirming the predominant disturbance of vertical gaze in this disease.

One or more other patients showing characteristic supranuclear ocular motor symptoms were documented with abetalipoproteinemia and sulfoaminoaciduria.

Significance to Biomedical Research and the Program of the Institute: The organization of a symposium on aldose reductase served the useful purpose of alerting other Institues to what has been a primary concern of NEI but also has wide implications for diabetic complications.

The note on gangliosidosis AB was prompted by the general lack of published information on the index case.

The report on the macula halo syndrome adds to the diversity of clinical signs in the Niemann-Pick syndrome and its specific ophthalmic manifestations.

The review of ocular motor disturbances in metabolic disease emphasizes the contrasting symptomology in Gaucher's disease and Niemann-Pick disease.

Proposed Course: Continued observation as opportunities arise.

NEI Research Program: Corneal Disease--Corneal Edema, Endothelial Dysfunction Dystrophies, and Inherited Disease (Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies)

Publications:

Cogan DG, Kinoshita J, Kador P, Datilis M, Robison G, Cobo M, and Kupfer C: Aldose reductase and complications of diabetes. Ann Int Med 101:81, 1984.

Cogan DG: Gangliosidosis AB. Neuro-Ophthalmology 4(1)65, 1984.

Cogan DG, Chu FC, Barranger JA, and Gregg RE: Macula halo syndrome, variant of Niemann-Pick disease. Arch Ophthamol 101:1698, 1983.

Cogan DG, and Chu FC: Supranuclear ocular motor symptoms in metabolic disease. Trans. Fifth Meeting Int'l Neuro-Ophthalmol Soc., Antwerp, Belgium, May 14-18, 1984 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00117-04 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oculomotor Disorders in Human Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G.Cogan	M.D.	Head, Section on Neuro-ophthalmology	CB, NEI
Others:	Fred C.Chu	M.D.	Senior Staff Fellow	CB, NEI
	Georgia A. Chrousos	M.D.	Senior Staff Fellow	CB, NEI
	Victor Matsuo	Ph.D.	Staff Fellow	CB, NEI
	James Carl	M.D.	Senior Staff Fellow	LSR, NEI

COOPERATING UNITS *(if any)* Mt. Sinai School of Medicine (A. Leist); Department of Mental Health, NIH (D. Pickar, G. Chrousos); Department of Neurology, Johns Hopkins Hospital (D. Zee); Department of Ophthalmology, Temple University (N. Schatz and P. Savino); Department of Ophthalmology, Georgetown University (J. O'Neill).

LAB/BRANCH
Clinical Branch

SECTION
Section on Neuro-ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Eye movements have been the chief emphasis of this past year's activities, and collaboration with other ocular motor research centers has been a major strategy. The miscellaneous observations can be represented only sketchily in a summary.

Congenital nystagmus was found to be associated at times with decreased vestibular gain. The mechanism of congenital nystagmus is most likely a "miswiring" at the mesencephalic level. Its varied forms can be simulated by the computer. The vertical saccades in Parinaud's syndrome are normal within their limited excursions. Ocular motor palsies in one eye can evoke horizontal oscillations in the non-paralyzed eye. Saccadic velocities were found to be a good index of drug sensitivity in normal persons and in patients with progressive supranuclear palsy.

Other observations included: documentation of ocular abnormalities in Turner's syndrome; a functional interpretation of head-turning in spasmus nutans; a graphic method of recording the Lancaster red-green test for ocular palsies; down-beat nystagmus precipitated by carbamazepine in a patient with the Arnold-Chiari syndrome; and the absence of the near-reflex in an otherwise healthy adolescent.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

Rex Cowdry	M.D.	Clinical Director	CPB, NIMH
Gordon Cutler	M.D.	Senior Investigator	DEB, NICHHD
Daniel Hommer	M.D.	Staff Psychiatrist	BPB, NIMH
Daniel Kenigsberg	M.D.	Medical Staff Fellow	DEB, NICHHD
D. Lynn Loriaux	M.D.	Clinical Director	DEB, NICHHD
Lance Optican	Ph.D.	Senior Staff Fellow	LSR, NEI
Maureen Polsby	M.D.	Medical Staff Fellow	ETB, NINCDS
Judith Ross	M.D.	Medical Staff Fellow	DEB, NICHHD
Alec Roy	M.D.	Visiting Associate	NSB, NIMH

Protocol Numbers: 77-EI-140 and 82-EI-78

Objectives:

1. The documentation of eye movement disorders in selected patients with neurologic disease.

2. The computerization of a library of video tapes and graphs selected for a research file.

Methods Employed: The clinical presentations are first recorded by video, then by infrared, electro-oculographic, or search coil techniques. The choice of the method depends on the cooperation of the patient and the type of movement to be recorded.

Major findings:

1. In collaboration with the Laboratory of Sensorimotor Research we have participated in a study of the vestibulo-ocular reflex in congenital nystagmus with the search coil technique. In three of five patients, the vestibulo-ocular gain was significantly less in the horizontal, compared to the vertical, direction. This decrease in gain was due in part to the instability of horizontal gaze and may have served to improve visual acuity during fixation.

2. Several studies have been carried out in collaboration with the Department of Neurology of Johns Hopkins Hospital. Computer simulation of the various wave forms in congenital nystagmus has supported the assumption of aberrant connections in the brain stem. The neural networks which maintain the eyes in eccentric gaze appear to be "miswired"; a negative feed-back loop has become positive. Additionally, the role of head shaking to improve vision in congenital nystagmus was found to depend on the presence or absence of the vestibulo-ocular reflex.

3. A study of vertical saccades in Parinaud's syndrome has revealed a number of deficits implying different types of abnormalities in ocular motor control. In some patients, we found normal velocities despite a restricted range of eye motion. Apparently, the vertical burst neurones can function normally within a limited range but lack the appropriate control signal when the eye moves beyond this range. In other patients, the vertical saccades simply fell short of the

target, but were otherwise normal for all positions. This is identical to the ocular motor deficit with experimental collicular-thalamic lesions of monkeys and suggests a failure in the generation of a neural signal appropriate for the actual position of the eyes in space.

4. The adaptation of the saccadic and pursuit movements has been analyzed in three patients with ocular muscle palsy. Especially noteworthy was the new finding that the pursuit system could adaptively increase its innervation to such a degree that the normal eye develops pendular oscillations. This suggests a potential mechanism for the creation of pendular nystagmus in patients.

5. In collaboration with the National Institute of Child Health and Human Development we have been able to study the ocular abnormalities in a cohort population of 30 patients with Turner's syndrome (chromosomal 45XO or 45XO/46XX mosaicism). Strabismus was present in a third of the patients. Other abnormalities included: ptosis, hyperteleorism, epicanthus, and antimongolian status. Ten percent of the patients also had a red-green color deficiency.

6. A graphic method of recording the Lancaster red-green test has enabled us to represent the relative muscle strengths of the ocular muscles by curves over the entire orbital range. This facilitates the diagnosis of ocular muscle deviation.

7. In children with spasmus nutans the head turn was found to be a compensatory mechanism which dampens the nystagmus and aids vision. The mechanism underlying this phenomenon was thought to be mediated by the vestibular or cervico-vestibular system.

8. In collaboration with NINCDS and the Mt. Sinai School of Medicine we are conducting eye movement studies (saccadic refixation, pursuit, and optokinetic nystagmus) on patients with progressive supranuclear palsy. The purpose is to monitor the progress of the disease and to determine the possible effects of drug therapies.

9. Two unique although unrelated observations were described in two patients: 1) downbeat nystagmus precipitated by carbamazepine (Tegretol) in association with the Arnold-Chiari malformation; and 2) the absence of the near reflex in an otherwise healthy adolescent.

Significance to Biomedical Research and the Program of the Institute: Observations of patients' signs and symptoms and their documentation are essential to the understanding of clinical manifestations of disease. The foregoing studies illustrate the fruitfulness of inter-Institute collaboration.

Proposed Course: To be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus

Publications:

Chrousos GA, Ross JL, Chrousos G, Chu FC, Kenigsberg D, Cutler G Jr, and Loriaux DL: Ocular findings in Turner's syndrome, a prospective study. Ophthalmology (in press).

Optican L and Zee D: A hypothetical explanation of congenital nystagmus. Biol Cybern 50:119, 1984.

Chu FC, Optican L, Zee D, Carl J, and Cogan DG: Upward saccades and Parinaud's syndrome. Abstract. Invest Ophthalmol Vis Sci. 25:263, 1984.

Chrousos GA, Roy A, Reingold DB, Hommer D, and Pickar D: Eye movement abnormalities in major depressive disorders. Psychiatry Research (in press).

Chrousos GA, Cowdry R, Reingold DB, and Chu FC: Downbeat Nystagmus and Oscillopsia associated with the use of Carbamazepine (Tegretol) in a patient with the Arnold-Chiari malformation. Ann Neurol (in press).

Cogan DG: Internuclear Ophthalmoplegia. Bull Soc Belge Ophtal 208-I:205, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00160-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Inattention After Posterior Cerebral Hemisphere Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David G. Cogan	M.D.	Head, Section on Neuro-ophthalmology	CB, NEI
Others:	Fred C. Chu	M.D.	Senior Staff Fellow	CB, NEI
	Georgia A. Chrousos	M.D.	Senior Staff Fellow	CB, NEI
	Jon Currie	M.D.	Visiting Scientist	CB, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Branch

SECTION
Section on Neuro-ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.45	.1

CHECK APPROPRIATE BOX(ES)
- ☒ (a) Human subjects
  - ☐ (a1) Minors
  - ☒ (a2) Interviews
- ☐ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In its incipient stages Alzheimer's disease may present diagnostic confusion by its focal visual symptoms. Tests with the Gunkel chromaticity method showed broad elevation of color thresholds in retinitis pigmentosa and defects in the orange-cyan bands with optic nerve lesions.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 83-EI-55

Objectives: To study residual visual function in patients with focal neurologic signs involving the visual system.

Methods Employed: Selected patients presenting with conspicious visual symptoms were subjected to appropriate tests including: visual acuity, visual fields, color vision, graphic tasks, constructional facility, and language tests. In most cases x-ray, CT scans, and PET scans are also available on these patients.

Major Findings: Noteworthy in this past year's experience has been the possibility to study several patients with Alzheimer's disease in whom the primary disturbance was one of visual incapacity. The visual symptoms were, in general, of two types: difficulty in spatial orientation and/or inability to interpret visual images.

Significance to Biomedical Research and the Program of the Institute: Although Alzheimer's disease is one of general mental deterioration, a few patients will present with focal symptoms suggesting brain tumor or vascular disease. Such patients may make repeated visits to the ophthalmologist or optometrist before the final diagnosis becomes apparent.

A comparative study of color vision in neurologic disease confirmed the simplicity and usefulness of the Gunkel chromaticity method. An incidental finding was the unexpected elevation of all color thresholds in retinitis pigmentosa and a predominant defect in the orange-cyan bands with optic nerve lesions rather than the expected red-green bands.

Proposed Course: To be continued.

NEI Research Program: Strabismus, Amblyopia and Visual Processing--Visual Processing and Amblyopia (Sensory Neuro-Ophthalmic Disorders)

Publications:

Chu FC, Reingold DB, Cogan DG, Hunt SM, and Young DH: Clinical Studies of Color Vision with Gunkel's Chromograph. Arch Ophthalmol 101:1232, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00161-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Congenital Nystagmus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Fred C. Chu	M.D.	Senior Staff Fellow	CB, NEI
Others:	Georgia A. Chrousos	M.D.	Senior Staff Fellow	CB, NEI
	David G. Cogan	M.D.	Head, Section on Neuro-ophthalmology	CB, NEI
	James Carl	M.D.	Senior Staff Fellow	LSR, NEI
	Lance Optican	Ph.D	Senior Staff Fellow	LSR, NEI

COOPERATING UNITS *(if any)* Department of Ophthalmology, Georgetown University (J. O'Neill); Childrens Hospital (Hammock, MK); Department of Ophthalmology, Johns Hopkins University Hospital (M. Lavery).

LAB/BRANCH
Clinical Branch

SECTION
Section on Neuro-ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
.55	.45	.1

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nystagmus in infancy presenting as spasmus nutans may signify a tumor in the region of the chiasm or optic nerves.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To characterize forms of congential nystagmus and to apply pharmacologic agents in their treatment.

Methods Employed: Documentation by video tape and graphic recording with use of infrared, skin electrodes, or search coil technique where appropirate.

Major Findings: The Neuro-Ophthalmic Section participated in a multi-institutional study of nystagmus in infancy due to an optic nerve or chiasmal glioma but masquerading as spasmus nutans.

Significance to Biomedical Research and the Program of the Institute: Clinically provides valuable information in helping to distinguish a progressive or threatening intracranial abnormality, which is potentially threatening, from a benign condition which requires no further investigation or treatment. Scientifically provides insights into possible mechanisms for generation of several forms of nystagmus.

Proposed Course: Project finished.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Disorders--Motor Neuro-Ophthalmic Disorders)

Publications:

Lavery M, O'Neill J, Chu FC, and Martyn LJ: Acquired nystagmus in early childhood: A presenting sign of intracranial rumor. Ophthalmology 91:5425, 1984.

O'Neill JF, Chu FC, Cogan DG, and Hammock MK: Acquired nystagmus in infancy: a benign or threatening condition? Two case reports of Russell's diencephalic syndrome. In Strabismus II, Reinecke R, editor. Philadelphia, Greene & Straaton (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00011-10 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pigment Dispersion With and Without Glaucoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Manuel Datiles	M.D.	Visiting Scientist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to compare patients with and without glaucoma having pigment dispersion syndrome. The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to developing glaucoma as well as add to understanding of the pathology of the disease.

Project Description:

Additional Personnel Engaged on Project:

Linda Wang	B.S.	Health Technician (Ophth.)	CB, NEI
Marvin Podgor	M.S.	Statistician	OBE, NEI
Paul Edwards	M.D.	Visiting Fellow	CB, NEI

Protocol Number: 76 EI 189

Objectives: (1) To compare patients with and without glaucoma having pigment dispersion by documenting and following the clinical features and course of their disease and by evaluating performance on a variety of diagnostic tests. (2) To determine the presence of abnormal aqueous humor dynamics in patients having pigmentary dispersion with and without glaucoma. (3) To compare pigment dispersion with and without glaucoma with respect to possible genetic markers. (4) To determine whether pupillary responses to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: A complete evaluation included the following examinations:

Complete family history with detailed pedigree
Best-corrected visual acuity with manifest refraction
Slit lamp biomicroscopy
Visual field examination (Goldmann I_{2e} and I_{4e})
Applantation Goldmann tension
Photography of iris color, iris transillumination and Kruckenberg spindle
A scan measurements, anterior chamber depth and anterior chamber volume measurements
Goniophotography
Static perimetry
Base-line tonography and water-drinking tonography one hour later, when indicated
Fasting blood sugar when indicated
Pupillography
Dilated ophthalmoscopic examination (2.5% phenylephrine and 1% cyclogel)
Stereophotographs of the optic nervehead

Major Findings:

1. This natural history study of pigment dispersion syndrome continues to uphold the finding that the majority of patients recruited appear to have a benign course and do not develop ocular hypertension or glaucoma.

2. It also appears that those patients who develop ocular hypertension and who demonstrate early field changes can be managed medically with control of intraocular pressure and reversal of early field loss. Patients who develop glaucoma do not appear to be more difficult to treat than patients with open-angle glaucoma.

3. A hereditary predisposition is present in some families. Consequently, family members should be alerted to this information and appropriately screened.

4. Cataract extraction in pigment dispersion syndrome and glaucoma may have an early beneficial effect on control of their intraocular pressure.

5. The entity of unilateral or assymetric pigment dispersion syndrome with little difference between the measurements of the two eyes is an interesting phenomenon which is being persued by follow-up studies.

6. In our series, retinal detachment does not appear to occur with any unusual frequency. History of peripheral retinal holes which are asymptomatic and non-progressive has been noted in two patients.

7. The data from this study have been computerized, and an in-depth analysis is underway.

Significance to Biomedical Research and the Program Institute: These data may make it possible to determine the risk of patients with pigment dispersion to developing glaucoma. Specifically, it may be possible to identify which features have predictive value in forcasting which patients having pigment dispersion will develop a visual field defect. In addition, the data will aid investigation of the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma.

Proposed Course: This project will be continued for three more years to obtain additional data that will add to knowledge about pigment dispersion syndrome.

NEI Research Program: Glaucoma--Other Glaucomas (Developmental, Congenital, and Infantile Glaucomas)

Publications:

Kaiser-Kupfer MI, Kupfer C, and McCain L: Asymmetric pigment dispersion. Trans Am Ophthalmol Soc LXXXI:310, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00060-08 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Visual Function and Ocular Pigmentation in Albinism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
Others:	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Rafael Caruso	M.D.	Expert	CB, NEI
	Linda Wang	B.S.	Health Technician	CB, NEI
	Patricia Mercer	B.S.	Clinical Technician	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.65	.35	.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Patients with hypomelanotic disorders such as ocular albinism, oculocutaneous albinism, Chediak-Higashi disease, Hermansky-Pudlak syndrome, and iris transillumination defects are being recruited to determine visual function in these conditions and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.

Project Description:

Other Professional Personnel Engaged on Project

Doris Collie	A.A.	Health Technician	CB, NEI
Mary Fuhrman		Health Technician	CB, NEI

Protocol Number: 76-EI-207

Objectives: The objectives of the study are (1) to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; (2) to correlate the amount of nystagmus with visual acuity and iris pigmentation; (3) to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; (4) to identify the heterozygous state in family members; and (5) to determine whether abnormalities of crossing of the optic nerve fibers can be correlated with the lack of pigmentation.

Methods Employed: The following examinations are performed:

- Complete family history with detailed pedigree
- Best-corrected visual acuity at near and distance with refraction
- Slit lamp examination
- Psychophysical testing including D-15 and Munsell 100 hue, rod and cone thresholds
- Dilated ophthalmoscopic examination
- Hair bulb incubation when indicated
- Photography to document hair color, eye color, iris transillumination, disc, and macula
- Visual evoked response
- Contrast sensitivity in selected patients

Examination of family members includes:

- Best-corrected visual acuity
- Slit lamp examination of iris
- Photography of iris transillumination
- Fundus examination when vision not corrected to 20/20

Major Findings:

Examination of patients and family members indicates that transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector. This finding is not associated with nystagmus.

Two patients have presented with marked iris transillumination, reduced pigmentation of the fundus, and no nystagmus, but decreased visual acuity which has improved in conjunction with an increase of the fundae pigmentation.

Visual evoked responses have been normal in some patients but in a subset of albinos appear to show evidence of abnormalities of crossing of optic nerve fibers at the chiasm. These findings are being pursued with more refined and updated equipment.

Significance to Biomedical Research and the Program of the Institute:

These data may allow identification of the carrier state in albinism, which would be of importance in genetic counselling. It may be possible to determine whether the development of the fovea is abnormal in albinism and if this is the cause of the decreased visual acuity in albinism or whether decreased visual acuity is secondary to hypopigmentation and the resultant light-scatter and glare. In addition, it will be possible to ascertain whether visual acuity improves with age and if this is correlated with changes in pigmentation.

Furthermore, studies are being conducted to verify the reported findings of abnormalities of the crossing fibers as measured by visual evoked responses and contrast sensitivity, degree of nystagmus, and amount of pigmentation.

Proposed Course: This project will be continued for five more years to obtain additional data.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00062-08 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Irido-Corneal-Endothelial (ICE) Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Manuel Datiles	M.D.	Visiting Scientist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.28	.23	.05

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was formerly titled "Progressive Essential Iris Atrophy." Patients are being recruited with progressive essential iris atrophy with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process and to investigate aqueous humor dynamics in both affected and unaffected eyes.

Project Description:

Additional Personnel Engaged on Project:

Linda Wang	B.S.	Health Technician (Ophth.)	CB, NEI
Paul Edwards	M.D.	Visiting Fellow	CB, NEI

Protocol Number: 76 EI 219

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, specular microscopy, physical correlates, and iris fluorescein angiography, when indicated to determine the role of the vasculature, are carried out in the primarily affected eye and the contralateral eye.

Methods Employed: During the course of the evaluation, the following procedures are performed:

Complete family history with detailed pedigree
Best-corrected visual acuity with manifest refraction
Slit lamp biomicroscopy
Visual field examination (Goldmann I_{2e} and I_{4e})
Photography of the iris and iris transillumination
Specular microscopy
Gonioscopy and goniophotography
Iris fluorescein angiography and photography (selected cases)
Baseline tonography
A complete medical and dental evaluation
Dilated ophthalmoscopic examination
Stereophotographs of the optic nervehead following dilation

Major Findings:

1. Seven patients with this rare condition have been recruited into the study and are being followed. Histopathologic and electron microscopic studies of iris and trabecular meshwork tissue have not provided any clues to the pathogenesis of the disease process.

2. An ultrathin corneal contact lens has been useful in patients with corneal bullae by preventing their rupture and controlling pain.

3. Although management of glaucoma and corneal abnormalities in the affected eye of patients with essential atrophy presents the major problem, our studies show subclinical involvement of the second eye including decreased outflow facility, iris transillumination, and corneal endothelial pathology.

4. Involvement of the contralateral eye, although subclinical, adds to the concept that this conditon may be appropriately included in the ocular neurocristopathies.

5. Due to advances in our understanding of the pathophysiologic mechanisms in this disorder, it is now referred to as the irido-corneal-endothelial (ICE) syndrome.

Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels, which may add to the understanding of the mechanism of glaucoma.

Proposed Course: The project will continue for four more years in an effort to obtain more data regarding the pathophysiology of this process.

NEI Research Program: Glaucoma--Other Glaucomas (Developmental, Congenital, and Infantile Glaucomas)

Publications:

Kupfer C, Kaiser-Kupfer MI, Datiles M, and McCain L: The contralateral eye in the irido-corneal endothelial (ICE) syndrome. Ophthalmology 90:1343, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00083-07 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Gyrate Atrophy of the Choroid and Retina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
Others:	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Francisco de Monasterio	M.D.	Head, Section on Visual Processing	CB, NEI
	Linda Wang	B.S.	Health Technician	CB, NEI

COOPERATING UNITS *(if any)*

Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland (David Valle, M.D.)

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.9	.4	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with gyrate atrophy of the choroid and retina are examined systematically to confirm the diagnosis. Skin fibroblasts of affected patients and family members are grown in tissue culture and assayed for ornithine aminotransferase activity. The results will be evaluated for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given a trial of pyridoxine to see if serum concentration of ornithine can be reduced, and, if so, the patient will be classified as a "responder," and treatment with pyridoxine will be continued. Nonresponder and responder patients will be placed on a low arginine, low protein diet with supplemental amino acids and observed for an arrest or improvement of their disease. If patients are not considered eligible for the diet or if they appear unable to comply with the dietary regimen they will be followed to record the natural progress of the condition. Patients with other forms of retinal degeneration, such as retinitis pigmentosa, fundus flavimaculatus, juvenile retinoschisis, are also examined and their courses are compared with gyrate atrophy patients.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

Paul Edwards	M.D.	Visiting Scientist	CB, NEI
Manuel Datiles	M.D.	Visiting Scientist	CB, NEI
Irene Ludwig	M.D.	Senior Staff Fellow	CB, NEI
Kent E. Higgins	Ph.D.	Expert	CB, NEI
Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI
Doris J. Collie	A.A.	Health Technician	CB, NEI
Mary E. Fuhrman		Health Technician	CB, NEI

Protocol Number: 78-EI-01

Objectives: (1) To determine the biochemical processes responsible for the elevated serum ornithine and the chorioretinal lesion that occurs in gyrate atrophy of the retina. (2) To determine which patients respond to pyridoxine treatment with a decrease in serum ornithine concentration. (3) To determine if treatment of "responders" with pyridoxine and/or dietary manipulation will arrest the progress of the retinal atrophy. (4) To study the natural history of these conditions if intervention is not undertaken and to determine the degree of heterogeneity.

Methods Employed: Patients suspected of having gyrate atrophy of the retina are examined according to a standard set of procedures to confirm the diagnosis. Plasma ornithine concentration is measured periodically. Punch biopsies of the skin are grown in tissue culture, and their enzymatic activity related to ornithine metabolism is measured. Complete evaluation of ocular function, including best-corrected visual acuity, Goldmann visual fields, color vision testing with HRR Nagel anomaloscope, D-15 and 100 hue test, cone thresholds, dark adaptation, electroretinograms, electro-oculogram, and contrast sensitivity are performed serially. Interferon and S antigen levels are determined.

Major Findings:

Gyrate atrophy, a rare autosomal recessive disorder is associated with hyperornithimia, overflow ornithenuria and a deficiency of activity of the mitochondrial enzyme ornithine- δ-aminotransferase.

Although rare, the condition has been described worldwide in all races. Twenty-two patients have been recruited and evaluated in our study. The ethnic origin has been varied. These included Scottish, English, Welsh, Portugese, Finnish, Lebanase, American Black, Asian Indian, German, and Israeli.

The age range of our patients has been from 2 years and 10 months to 65 years. The earliest clinical and electrophysiologic features were documented in our two youngest patients. Noteworthy is the minimal evidence of clinical

retinal changes when significant reduction of rod and cone function is seen by electroretinographic studies.

Clinical and biochemical evidence of genetic heterogeneity is present. Less than 10% of patients have been reported to have a 30-50% decrease of plasma ornithine following treatment with vitamin B_6. Only one of our patients showed an in vivo response to this treatment.

Since arginine is the precursor of ornithine in the metabolic pathway of ornithine metabolism, a dietary intervention study limiting arginine has been undertaken. Fourteen patients were placed on a low protein (low arginine diet). All sustained a significant reduction of ornithine while in the hospital. However, the diet was discontinued in four Finnish patients following their discharge because of poor compliance (and the long distance) and one American patient because of emotional and social factors. Nine patients remain on the diet, two with good control, six with fair control, one with poor control. Ophthalmologic evaluations are performed on all patients every six months.

The two patients with the best biochemical control showed evidence of improved visual function. One patient on the diet for months was found to show an improvement in dark adaptation, averaged ERG, and color vision after 13.5 months. This improvement was sustained for 30 months and then showed a small but definite reduction in the ERG amplitude. The second patient, after 15 months on the diet and with lowered plasma ornithine levels showed progressive improvement in visual field and color vision testing. The third patient, despite good control, was stable for 36 months, but has deteriorated for the last 12 months. It is noteworthy that she was the eldest and had the poorest control at the outset. Other patients followed for varying lengths of time appear currently stable.

All but one patient over age 11 have had cataracts, which are seen in the posterior capsule, are progressive, and show a uniform histologic picture.

Systemic findings in this condition are subclinical and include abnormalities of hair, EEG abnormalities, tubular aggregates in muscle, and rarely muscle weakeness.

Significance to Biomedical Research and the Program of the Institute:

Gyrate atrophy of the retina is the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical marker and concomitant enzyme defect have been demonstrated. The study will test the efficacy of treatment for this blinding eye disease and serve as a model for the investigation of other genetically determined retinal degenerations. The two young patients will enable the best opportunity for the evaluation of diet control.

Proposed Course: This project will be continued for three more years to further assess the knowledge of reduced ornithine in halting the chorioretinal degeneration.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NIH Interinstitute Medical Genetics Program: The Genetics Clinic

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. Name (Last, First, Middle Initial) | Laboratory and Institute Affiliation | Professional Degree | Staff Position Title | Laboratory and Institute Affiliation)

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
Others:	Paul Edwards	M.D.	Visiting Fellow	CB, NEI
	Irene Ludwig	M.D.	Staff Ophthalmologist	CB, NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Linda Wang	B.S.	Health Technician (Ophth.)	CB, NEI

COOPERATING UNITS (If any)

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.4	.2	.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Interinstitute Medical Genetics Program and the Genetics Clinic, supported by the Clinical Center, offer a multidisciplinary approach to patients with genetic disease (Z01 CP 05139-04 CEB). Involved in the program are researchers from all Institutes. Patients evaluated in the clinic represent a broad spectrum of genetic disease. During the last year, approximately 423 individuals were seen, representing approximately 100 different disease categories. Due to the high frequency of ocular involvement in many of the cases, almost all the patients were evaluated by Clinical Branch staff or were discussed in consultation. The Clinic serves as a source of interesting case material concerning patients with inherited or developmental abnormalities of the visual system.

In addition to the Genetics Clinic, patients are seen for genetic consultation at the Maryland School for the Blind. This experience has resulted the recruitment of patients into Clinical Branch protocols.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: Interinstitute Medical Genetics Program

Objectives: (1) To evaluate patients with ocular abnormalities associated with genetic disease in the context of a multidisciplinary approach to the patient. (2) To provide genetic counseling to patients at risk for inherited ocular disease. (3) To recommend and advise appropriate evaluation for the ocular problem. (4) To provide training in the diagnosis, counseling, and treatment of individuals with or at risk for genetic disease and in the research approach to genetic disease.

Methods Employed: Referred patients are examined, and the appropriate ophthalmologic work-up is recommended as necessary for diagnostic purposes.

Major Findings:

1. Iris nodules are commonly seen in the classic cases of neurofibromatosis, seen less frequently in patients with less well-defined disease, and not seen in patients with acoustic neuroma.

2. Osteogenesis imperfecta patients plus controls examined number 109. Our initial studies showed decreased ocular rigidity and an updated analysis confirms decreased ocular rigidity, which is correlated with blueness of sclerae.

3. Families with aniridia of the sporadic and dominant form are being evaluated to determine the presence of chromosome deletions and catalase deficiency. A patient with aniridia, mental retardation and genitourinary abnormalities has been shown to have a deletion of 11p and a reduced activity of catalase.

Significance to Biomedical Research and the Program of the Institute:

Genetic and developmental anomalies of the eye are a major cause of blindness and visual disability, representing about 35% of the causes of blindness in developed nations. Involvement with the Interinstitute Medical Genetics Program will afford a systemic approach to these conditions and to other conditions associated with other genetic diseases.

Proposed Course: The project is in a growth phase and will be expanding in future years.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Kaiser-Kupfer MI, Podgor MJ, McCain L, Kupfer C, and Shapiro JR: Correlation of ocular rigidity and blue sclerae in osteogenesis imperfecta. Trans Am Ophthalmol Soc (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00172-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Senile Macular Degeneration

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics	CB, NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Monique Roy	M.D.	Visiting Scientist	CB, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.43	0.43	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will determine if patients with severe visual loss because of senile macular degeneration in one eye and with good vision in the second eye can be protected from severe visual loss in the good eye by the administration of vitamin E and vitamin C when exposure of the retina to light below 500 nanometers is diminished. The recruited patients will be randomly assigned either to a treated or an untreated control group and examined at four-month intervals. Follow-up will continue for five years, unless an early beneficial or detrimental effect causes the study to be terminated in less than five years.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 82-EI-169

Objectives: To determine if supplemental vitamin E and vitamin C under diminishing exposure of the retina to light below 500 nanometers are effective in preventing severe visual loss in the good eye of patients with severe visual loss in the other eye due to senile macular degeneration (SMD).

Methods Employed: Patients are examined to determine eligibility for the study. Eligible patients, if they agree to participate, are assigned by randomization to either a treated or untreated control group. Both groups are instructed to use special glasses all the time outside and inside when in the presence of fluorescent and bright lights.

Patients will be given a standard eye examination every four months, including visual acuity testing, and will receive a resupply of vitamin capsules. Stereo fundus photographs of each macula will be taken once a year. The data will be evaluated every four months by a data monitoring and safety committee. The end point for the study will be visual acuity of 20/100 or less in the initially better eye because of disciform or atrophic degeneration of the macula.

If the treated group has more visual loss, or more adverse effects than the control group, the study will end.

Major Findings: One hundred and ten patients have been examined to determine their eligibility, and 49 patients have now been randomized to treatment. No intolerance to the pills or glasses has been reported. One patient has died from myocardial infarction, which appeared unrelated to the protocol treatment. One patient developed in the initially good eye a serous detachment of the macula without any evidence of subretinal neovascularization on fluorescein angiography; vision has remained stable at the 20/40 level. Another patient developed in the initially good eye a small area of para-macular subretinal neovascularization which was successfully treated with krypton laser photocoagulation. Vision in that eye has remained stable at the 20/40 level.

Significance to Biomedical Research and the Program of the Institute: Senile macular degeneration is the leading cause of newly registered blindness in the white adult population in the United States. Patients who have lost central vision in one eye because of SMD lose central vision in the other eye at a rate of 10% per year. Although epidemiologic studies have tried to identify risk factors associated with SMD, the cause of SMD remains unknown. There is some evidence that the retina is more sensitive to light damage for wavelengths below 500 nanometers. The generation of free radicals in the retina as a result of certain wavelengths of light may be a major factor in the pathogenesis of SMD. This study will obtain data that are expected to enhance our understanding of SMD while at the same time investigating a method for treating it.

Proposed Course: For a 50% effect of treatment at 5 years, 86 patients will be needed both in the treated and control groups. Two hundred twenty-five patients will be recruited for this protocol to take into account losses because of death or inability to follow-up. Recruitment has increased significantly in the last few months following announcement of the study in various newspapers and magazines.

NEI Research Program: Retinal and Choroidal Disease--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00188-01 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Documentation and Monitoring of Opacities in the Human Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Manuel B. Datiles	M.D.	Visiting Scientist	CB, NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Robert Sperduto	M.D.	Epidemiologist	OBE, NEI
	Peter Kador	Ph.D.	Chemist	LVR, NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Ted Gancayco	B.S.	Chemist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
.45	.3	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are in the process of developing the means to monitor and document opacities in the human lens using different systems. We have recently acquired a Scheimpflug camera, which is presently the best means of documenting cataracts. However, we are exploring other means such as ultrasonography, specular microscopy, and nuclear magnetic resonance.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 84 EI-132

Objectives: The objective of this project is to formulate means to document cataract formation and progression. This is an important step prior to clinical trials of drugs that are claimed to prevent cataract prevention and progression.

Methods Employed: Complete ophthalmologic examination. We will do Scheimpflug photography of the cataracts.

Major Findings: The present system of photography and image analysis is found to be inadequate. We are designing an image analysis system which will circumvent some of the problems related to film processing and developing. We have also found a possible population group for future studies of presenile cataracts.

Significance to Biomedical Research and the Program of the Institute: Monitoring and documentation of human cataracts is a crucial step towards the ultimate testing of several medications which are believed capable of preventing the progression of human cataracts. This is also important in being for categorizing types of cataracts in various parts of the world and correlating them with the physical and genetic factors within a certain geographical region.

Proposed Course: We will continue the search for the ideal method of documenting and monitoring human cataracts. Improvement in the present system of lens photography, such as Sheimpflug, as well as exploration of possible applications of new technological advances will be pursued. Identification of appropriate population groups to be studied will also be pursued.

NEI Research Program: Cataracts--Epidemiology of Cataracts

Publications:

Stevens R, Datiles M, Srivastava S, Ausari N, Maumenee AE, and Stark WJ: Invest Ophthalmol Vis Sci 25a(Suppl):136, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 000187-01 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effects of Corneal Contact Lenses on the Cornea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Manuel B. Datiles	M.D.	Visiting Scientist	CB, NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
	Linda Wang		Health Technician (Ophth.)	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.45	.3	.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Short- as well as long-term effects of contact lens wear on the cornea are being investigated. Changes in corneal curvature, changes in corneal epithelial morphology and changes in corneal endothelial cell morphology are being studied by specular microscopy.

These data will help us understand the dynamics involved in the interaction between a contact lens and the cornea, the risks involved to corneal tissues, and how a systemic or local disorder may increase these risks.

Project Description:

Additional Personnel Engaged on Project:

Ted Gancayco	B.S.	Chemist	CB, NEI

Protocol Number: 84 EI-133

Objectives: The objective of this project is to investigate the effects of contact lens wear on corneal tissues. We also will study factors which increase or decrease the potential risk of injury to corneal tissues with contact lens wear.

Methods Employed: Complete history, ophthalmologic examination, photography, keratometry and specular microscopy will be employed.

Major Findings: We have found that diabetes may increase the risk of complications from contact lenses in the first six months of wear. In addition, we have found some changes in the corneal endothelium after long-term wear of contact lenses. This is being studied further.

Significance to Biomedical Research and the Program of the Institute: Contact lenses are commonly used for correction of errors of refraction as well as for therapy. However, our knowledge of the interaction of contact lenses with the cornea and tears is as yet imperfect. In addition, risks associated with wearing contact lenses are also poorly understood. The understanding of the interaction between contact lenses and corneal tissues will allow us to determine why some patients cannot wear contact lenses and may help avoid some of the complications associated with contact lens wear.

Proposed Course: The following studies are in progress or proposed for next year. (1) Continued recruitment and examination of patients who have worn hard and soft contact lenses for prolonged periods of time. (2) Continued recruitment and examination of normal volunteers for comparison. (3) Recruitment of patients who for one reason or another, have failed to wear contact lenses or developed serious complications from contact lens wear.

NEI Research Program: Corneal Diseases--Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Disease

Publications:

Datiles MB, Kracher G, Stark WJ, Maumenee AE, and Gordon A: Invest Ophthalmol Vis Sci 24a(Suppl):79, 1983.

Fagadau W, Kracher G, Stark W, Datiles M, and Maumenee AE: Extended wear contact lenses in aphakic patients with filtering blebs. Ophthalmology 24b(Suppl):108, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT | Z01 EY-00084-06 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension

PRINCIPAL INVESTIGATOR: List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Carl Kupfer	M.D.	Director	NEI
Others:	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI
	Lessie McCain	R.N.	Clinical Technician	CB, NEI
	Manuel B. Datiles	M.D.	Visiting Scientist	CB, NEI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.95	.65	.30

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

With recent embryological research indicating the role of the neural crest in contributing to all connective tissues anterior to the lens epithelium, the group of developmental anomalies of the anterior chamber with glaucoma or ocular hypertension is being reviewed.

Project Description:

Additional Personnel Engaged on Project:

Linda Wang	B.S.	Health Technician (Ophth.)	CB, NEI
Paul Edwards	M.D.	Visiting Fellow	CB, NEI

Protocol Number: 77 EI 119

Objectives: The object of this study is to determine whether congenital and/or developmental anomalies of the anterior chamber are related to faulty migration or terminal differentiation of neural crest tissue.

Methods Employed: Patients of all ages with congenital and/or developmental anomalies of the anterior chamber are being examined to determine involvement of cornea, trabecular meshwork, iris stroma, lens, and ciliary body. When intractable glaucoma that cannot be controlled with medication is present, surgery will be performed and the specimens examined histologically. When the lenses become cataractous, cataract extractions are performed and the lens epithelium grown in tissue culture.

In addition, we have recently started to study a strain of guinea pigs that develops congenital cataracts, agnathia, and bone formation intraocularly. Biochemical and histologic studies to characterize these congenital anomalies are being done.

Major Findings: It appears that in this group of anomalies of anterior chamber development there are pathological changes in one or several tissues derived from neural crest. These include corneal stroma, corneal endothelium, anterior iris stroma, Descemet's membrane and trabecular meshwork endothelium. In the guinea pig studies, preliminary findings show that the defect is an autosomal dominant inherited trait. The cataracts are nuclear and are found at birth. Bone formation intraocularly is not constant. Agnathia is found in stillborn animals. Biochemically, there is loss of a beta crystallin band and appearance of a gamma crystallin band. Histologically, there is failure of elongation of lens fibers, an abnormal denucleation process, poor formation of the nucleus and abnormally thin posterior capsule.

Significance to Biomedical Research and the Program of the Institute: A better understanding of the pathogenesis of these glaucomas may help in improving diagnosis and treatment.

Study of the guinea pig cataracts and associated anomalies may help us in understanding how congenital cataracts are formed in humans and what role the neural crest plays in cataract formation as well as the agnathis and abnormal bone formation.

Proposed Course: Patients with other anomalies of the anterior chamber including congenital cataracts will be examined for abnormalities in tissue derived from neural crests.

We will continue studies on the guinea pigs with congenital cataracts and its etiology as well as any relationship with neural crest abnormalities.

NEI Research Program: Glaucoma--Other Glaucomas (Developmental, Congenital, and Infantile Glaucomas)

Publications:

Kupfer C, Datiles M, Kaiser-Kupfer MI: Development of the anterior chamber of the eye: Embryology and clinical implications. In Basic Aspects of Glaucoma Research. Lutzen-Drecoll E, editor. Stuttgart, F.K. Schattauer Verlag, 1982, pp. 35-38.

Datiles M, Stone SH, Hu T-S, Ambaugh P, Zigler SJ, Kinoshita JH: Congenital cataracts in guinea pigs. Invest Ophthalmol Vis Sci 25a(Suppl):146, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00162-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Vitreous Fluorophotometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Monique Roy M. D. Visiting Scientist CB, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.50	0.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vitreous fluorophotometry will be performed in patients with diabetes mellitus without retinopathy, patients with diabetes mellitus with nonproliferative retinopathy, and normal volunteer subjects, age-, number-, and sex-matched to the patients. The amount of fluorescein leakage into the vitreous of patients will be compared to that of the normal subjects. Correlations with other features of diabetes, such as the quality of diabetic control, the existence of subclinical neuropathy and nephropathy, and others will be sought.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 82-EI-196

Objectives: To determine if fluorescein leakage as detected by vitreous fluorophotometry is one of the earliest signs of diabetic retinopathy, if it is influenced by the metabolic control of the diabetic state, and if it is associated with early signs of alteration of nerve and kidney function.

Methods Employed: Insulin-dependent diabetics with no or little background diabetic retinopathy are recruited. Eligible patients, if they agree to participate and have no known allergy to the fluorescein dye, receive an intravenous injection of fluorescein, followed by measurements of fluorescein leakage into the vitreous using the fluorotron master. A detailed physical examination is performed. Blood and urine are collected to assess the metabolic control of the diabetes, renal function, and different biological parameters.

The amount of fluorescein leakage into the vitreous of diabetic patients is compared with that of normal volunteers matched with the diabetics for age, sex, skin pigmentation, and iris color.

Major Findings: Twenty eight diabetics (14 males and 14 females) have been enrolled in the study. Twenty one normal subjects have also been recruited. Data will be analyzed as soon as all normal subjects have been recruited.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is the commonest cause of new blindness in the United States among people between the ages of 20 and 55. Although carbohydrate intolerance appears to be the major systemic abnormality, the precise cause of the onset and development of the retinal vascular changes is poorly understood. An early sign of vascular damage may be increased permeability of the retinal vessels as measured with vitreous fluorophotometry. Such knowledge would not only be valuable in itself but may also be extremely useful in clinical trials to rapidly assess the effects of drugs or of tighter metabolic control in preventing the microvascular complications of diabetes.

Proposed Course: The fluorotron master has proven to be easy to use clinically. It is hoped that its clinical usefulness will be fully determined when this matched study is completed.

NEI Research Program: Retinal and Choroidal Disease--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 000198-01 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Sorbinil Retinopathy Trial

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Monique Roy	M.D.	Visiting Scientist	CB, NEI
Others:	Manuel Datiles	M.D.	Staff Ophthalmologist	CB, NEI
	Oscar Cuzzani	M.D.	Visiting Scientist	CB, NEI
	James Carl	M.D.	Senior Staff Fellow	CB, NEI

COOPERATING UNITS *(if any)*

Division of Diabetes, Endocrinology, and Metabolic Diseases, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH (R. Silverman)

LAB/BRANCH

Clinical Branch

SECTION

Section on Ophthalmic Genetics and Pediatric Ophthalmology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.35	1.25	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral sorbinil, an aldose reductase inhibitor, will be administered in a double-masked randomized trial to diabetics with no or minimal diabetic retinopathy. This will be done to evaluate the effects of sorbinil on the development of diabetic retinopathy and further investigate the safety and toleration of sorbinil. The study will be conducted simultaneously in 12 research centers in the USA.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 83-EI-155

Objectives: In the presence of hyperglycemia, the enzyme aldose reductase becomes activated and glucose is converted to sorbitol through the "polyol" pathway. Intracellular accumulation of sorbitol may be responsible for the selective loss of pericytes seen early on in diabetic retinopathy. The objective of the study is to determine whether sorbinil, an aldose reductase inhibitor, may prevent the development of diabetic retinopathy.

The study will further evaluate the safety of sorbinil.

Methods Employed: Seventy patients, aged 18 to 56, with Type I insulin-dependent, ketosis-prone diabetes diagnosed before age 41, having had 1 to 15 years of chronic insulin therapy and showing less than 5 microaneurysms in either eye on DRS 7 stereo fundus photographs will be recruited. Female patients must either be postmenopausal, or surgically sterilized, or have an intrauterine device in place. Patients found eligible by the Data Coordinating Center at Harvard Medical School, if they agree to participate will be randomly assigned to oral sorbinil (250 mg) or placebo for a period of two and one-half years. Efficacy of the drug will be evaluated from DRS 7 field fundus photographs by the Photography Reading Center located at the University of Wisconsin in Madison.

In patients who enter the study without clinical signs/symptoms of peripheral neuropathy, the efficacy of the drug in preventing the development of diabetic neuropathy will be evaluated from the neurological examination, the clinical neuropathy questionnaire, and the nerve disability score.

Changes in retinopathy and neuropathy from baseline to the 12-, 21-, and 30-month evaluations will be compared between treatment groups.

Repeated physical examinations, blood tests, and eye examinations will be done to monitor the safety of the drug.

Major Findings: Thirty seven diabetics have been screened and 15 have been found eligible. Three eligible patients withdrew from the study. For 12 patients a decision on eligibility is still pending. Ten patients have been randomized.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is the commonest cause of new blindness in the USA among people between the ages of 20 and 55. At the present time there is no known way of preventing the development of diabetic retinopathy in the diabetic patient.

A role for the polyol pathway in the development of diabetic complications is based on several lines of evidence. Among them, there is a causal relationship between elevated polyol pathway products in the lens and subsequent cataract development in diabetic animal models. In diabetic patients, sorbinil has already been shown to improve defective motor and sensory nerve conduction.

Aldose reductase can be demonstrated in the pericytes of human retinal capillary by immunohistochemical techniques, thus suggesting that the polyol pathway could play a role in the early pathogenesis of diabetic retinopathy (i.e. mural cell loss).

Proposed Course: We expect that the seventy patients will be recruited by the end of 1984.

NEI Research Program: Retinal and Choroidal Disease--Diabetic Retinopahy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00092-06 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert B. Nussenblatt M.D. Head, Section on Clinical Ophthalmic Immunology CB, NEI

COOPERATING UNITS *(if any)*
Bureau of Biologics, FDA (Kamal Mittal, Ph.D.)

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.03	0.03	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with ocular toxoplasmosis, pars planitis, sarcoidosis, Behcet's disease, chorioretinitis of unknown origin, and birdshot retinochoroidopathy are being studied to determine the phenotype frequency of the HLA, ABO, and B-cell alloantigens. Because the B-cell alloantigens or DF antigens are thought to play a role in the immunologic response to antigens, these findings will complement other immune uveitis studies being simultaneously carried out.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 79-EI-48

Objectives: To determine whether patients with ocular inflammatory disease manifest specific HLA or B-cell alloantigens more frequently than the average population.

Methods Employed: Heparinized blood samples from patients are subjected to microcytotoxic tests to determine the HLA and B-cell antigens. The ABO system is evaluated utilizing an anti-sera method.

Major Findings: HLA-B8 has been found to be associated with iridocyclitis in black Americans. This antigen has been associated with a wide range of autoimmune diseases, and its presence in patients with this disorder strongly suggests a similar mechanism for this disease. Eighty percent of the birdshot retinochoroidopathy patients tested were positive for HLA-A29. The p .0001, with the computed relative risk also 50, is one of the highest recorded.

Significance to Biomedical Research and the Program of the Institute: The role of HLA and B-cell alloantigens in the immune response is only beginning to unfold. This study will indicate whether these alloantigens play a role in the ocular immune response.

Proposed Course: This study will continue so that sizeable populations of various ocular immune entities can be studied. An increased number of patients with a variety of ocular inflammatory conditions are being sought.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Mittal KK, Ryan S, Green WR, and Maumenee AE: Birdshot retinochoroidopathy--an association with HLA-A29 and immune responsiveness to retinal S-antigen. Am J Ophthalmol 94:147, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 EY 00075-06 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Functions in Ocular Diseases of Obscure Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
	William Leake	M.S.	Biologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vitro cellular immune functions and lymphocyte subsets are being studied in a masked method in patients with ocular toxoplasmosis, pars planitis, Behcet's disease, ocular sarcoid, birdshot retinochoroidopathy, geographic choroiditis, and chorioretinitis of unknown origin. Crude ocular antigens, as well as the purified uveitogenic soluble antigen (S-antigen) of the retina, are being used in a lymphocyte microculture technique to evaluate the presence of cellular immune memory to ocular tissues. A subgroup of patients with posterior uveitis has been identified as having this immunologic memory. Lymphocyte subsets in the blood and in the eye are being defined in these patients by monoclonal antibodies. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 79-EI-49

Objectives: The objective of this study is to investigate several immunological factors in ocular inflammatory disease and how they may relate to the course and chronicity of this disease. The identification of groups with specific immunologic alterations provides us with a more rational approach to therapy.

Methods Employed: The ophthalmic examination of all patients includes slit lamp examination, visual field tests, electroretinogram, and fluorescein angiography. Lymphocyte cultures are prepared using the microculture technique, to assess evidence of cellular immune memory to purified human and bovine S-antigen, which is considered to be the in vitro equivalent of the anamnestic response in vivo. Monoclonal antibodies to T-cell subsets, in conjunction with the fluorescence-activated cell sorter, are being used in an attempt to identify alterations in lymphocyte subgroups. Staining of ocular tissue using the avidin-biotin methodology is being utilized.

Major Findings: A subpopulation of patients with ocular inflammatory disease manifested a positive "memory" response to the S-antigen. Positive responders appear to be those with active or inactive retinal lesions, and patients with various diseases were found to respond. It therefore appears that similar immune groups are present in different clinical entities.

Some patients with posterior uveitis respond to crude retinal extracts but not to the S-antigen, indicating the possible role of other retinal antigens still to be purified. All patients with birdshot retinochoroidopathy that were tested manifest cell-mediated responses to either the S-Ag or crude retinal antigens.

The evaluation of an eye with sympathetic ophthalmia using monoclonal antisera to various immune cells demonstrated mostly the inducer fraction of T-lymphocytes in the eye. The large cells found in the Dalen Fuchs nodules obtained positively for macrophage markers.

A new uveitis entity, subretinal fibrosis with uveitis, was described. This relentless process, minimally responsive to anti-inflammatory activity, has striking retinoscopic changes and no known systemic association. Patients appear to be responding to the S-Ag in in vitro testing.

Significance to Biomedical Research and the Program of the Institute: Uveitis is the cause of 10 percent of visual impairment in the United States. This is the first time that patients' immune cells have been shown to manifest cellular immune memory to a purified retinal antigen and that alterations in suppressor cells are also present.

The grouping of patients with uveitis on the basis of specific immunologic functions or alterations may provide a more rational basis upon which to develop specific immunotherapy. Elucidation and treatment of inflammatory conditions of the eye are major interests of the NEI.

Proposed Course: This continuing study will focus on the posterior uveitic entities in order to investigate further the role of the S-antigen in each of these and what, if any, role abnormal suppressor cell activity may play.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Mittal KK, Ryan S, Green WR, and Maumenee AE: Birdshot retinochoroidopathy--an association with HLA-A29 and immune responsiveness to retinal S-antigen. Am J Ophthalmol 94:147, 1982.

Nussenblatt RB, Salinas-Carmona M, Leake W, and Scher I: T-lymphocyte subsets in uveitis. Am J Ophthalmol 95:614, 1983.

Palestine AG: In Current Therapy in Allergy, Immunology, and Rheumatology, Fauci, AS, editor. Philadelphia, BC Decker (in press).

Nussenblatt RB: In Biomedical Foundations of Ophthalmology, Duane TD, Jaeger ED, Friedlaender M, editors. Philadelphia, JB Lippincott (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00094-06 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Mechanisms in Experimental Autoimmune Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Igal Gery	Ph.D.	Visiting Scientist	LVR, NEI
	William Leake	M.S.	Biologist	CB, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.15	2.06	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lewis rats and non-human primates, immunized at a site distant to the eye with the retinal soluble antigen (S-antigen) in complete Freund's adjuvant, develop experimental autoimmune uveitis (EAU). Lymph node cells and peripheral lymphocytes from immunized animals manifested significant cellular immune responses measured by the lymphocyte culturing technique. Cyclosporine, a drug with specific anti-T-activity, has been found to be exceptionally effective in protecting rats with EAU, and suppressor cells potentially play a role in this protective mechanism. As well, the inducer cell T-cell fraction in the lymph node appears to be most susceptible to cyclosporine therapy. Attempts at local immunosuppressive therapy in order to prevent EAU have begun.

Project Description:

Other Professional Personnel Engaged on Project:

Rashid Mahdi	Biological Aid	CB, NEI
Francois Roberge	Guest Researcher	CB, NEI

Objectives: We have previously reported that experimental uveitis may be induced in animals by immunization with a purified component of the retina (S-antigen). This study is designed to elucidate the basic immunologic mechanisms of this laboratory model for uveitis and how this model may be altered or regulated.

Methods Employed: Lewis rats are immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad. After two to four weeks, lymph node or peripheral blood cells are collected and used for several cellular immune studies. Lymphocyte cultures are prepared in microtiter plates and are stimulated with S-antigen as well as other antigens. Lewis rats immunized with the S-antigen are "protected" by daily injections of cyclosporine. Antibodies are evaluated by gel diffusion, ELISA, and indirect hemagglutination techniques.

Major Findings: Animals immunized with S-antigen develop obvious clinical anterior and posterior uveitis, which is confirmed by histology. Animals with ocular disease manifest significant cellular immune memory responses when measured by lymphoproliferative techniques.

A comparison was made between the in vitro proliferative responses and lymphocyte subsets in S-Ag immunized rats treated with cyclosporine and treated with placebo. A kinetic study was performed in which blood, lymph node, and spleen lymphocytes were cultured 3.5, 7, 10, and 14 days after immunization, the latter being the peak of the ocular inflammatory response. The inducer cell subset of T-cells in the lymph node appeared to be most affected by daily cyclosporine (2 mg) therapy. The proliferative responses in the lymph node of rats treated with cyclosporine were decreased, and the significant proliferative responses to the S-antigen, form in the blood of untreated rats, were not seen in the CsA group. These data would support the concept that CsA's mode of action may be through the inhibition of the normal T-cell recruitment of immunoreactive cells.

Ongoing experiments are evaluating the possibility that local cyclosporine therapy may positively alter EAU's course. Though topical medication may be effective, the role of systemic uptake of the drug still needs to be critically evaluated.

Intraocular placement of S-Ag preliminarily does not appear to induce a tolerant state in rats as might be predicted by experiments concerning ACAID. These animals can be immunized systemically and develop ocular disease.

Significance to Biomedical Research and the Program of the Institute: Experimental autoimmune uveitis is the first uveitis model utilizing a purified retinal antigen. The mapping out of its immune mechanism may lead to an improved

understanding of human ocular inflammatory disease. Immunoregulatory models developed in this system including cyclosporine, will be utilized in future human clinical trials.

Proposed Course: To describe fully and underlying immune events in this disease and to develop a successful protocol dealing with either specific or nonspecific suppression of the disease.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Salinas-Carmona MS, Nussenblatt RB, and Gery I: Experimental autoimmune uveitis in the athymic nude rat. Eur J Immunol 12:480, 1982.

Nussenblatt RB, Salinas-Carmona MC, Waxman BH, and Gery I: Cyclosporin A: Cellular immune alterations in S-antigen induced experimental autoimmune uveitis. Int Arch Allergy Immunol 70:289, 1983.

Salinas-Carmona MC, Nussenblatt RB, and Gery I: Role of T-lymphocytes in the induction of experimental autoimmune uveitis in rats. In 3rd International Conference of Immunology and Immunopathology, Silverstein A, O'Connor R, editors. New York, Masson Publishing (in press).

Gery I, Robinson WG, Shichi H, El-Saied M, Mochizuji M, Nussenblatt RB, and Williams RM: Differences in susceptibility to experimental autoimmune uveitis among rats of various strains. In 3rd International Conference of Immunology and Immunopathology, Silverstein A, O'Connor R, editors. New York, Masson Publishing (in press).

Nussenblatt RB, Rodrigues MM, Salinas-Caroman MC, Leake WC, Franklin PE, Waksman B, Wacker WB, and Gery I: Cyclosporin A-induced clinical and immune alterations in experimental autoimmune uveitis. In 3rd International Conference of Immunology and Immunopathology, Silverstein A, O'Connor R, editors. New York, Masson Publishing (in press).

Nussenblatt RB, Silverstein A: Immunological ocular diseases, In The Autoimmune Diseases, Rose NR, Mackay IR, editors. Academic Press (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00115-04 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclosporine Therapy in Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Rafael C. Caruso	M.D.	Expert	CB, NEI
	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB, NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR, NEI
	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.50	2.00	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cyclosporine, an endecapeptide fungal product with specific anti-T-cell characteristics, will be administered to patients with sight-threatening ocular inflammatory disease of non-infectious origin who have failed on either corticosteroid or cytotoxic agent therapy. This will be done to test cyclosporine's efficacy in the treatment of uveitis. Another arm of this study is a randomized double-masked study which will evaluate the effectiveness of cyclosporine therapy to that of systemic corticosteroid administration.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project:

Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
Leslie S. Fujikawa	M.D.	Senior Staff Fellow	CB, NEI
William Leake	M.S.	Biologist	CB, NEI

Protocol Number: 81-EI-33

Objectives: Cyclosporine, an endecapeptide obtained from fungi, has been shown to have specific anti-T-cell activity (Transplantation Proc 12:234, 1980). We have reported cyclosporine's exceptional effectiveness in preventing the induction of S-antigen autoimmune uveitis in rats, as well as inhibiting the disease once immunization has occurred (J Clin Invest 67:1228, 1981). The goal of this study is to test cyclosporine's efficacy in treating patients with bilateral sight-threatening posterior uveitis of an autoimmune nature.

Methods Employed: Patients 18 years of age or older, of either sex (females not pregnant), who have not done well on more conventional medical therapy were admitted to this study. All patients will have a bilateral sight-threatening uveitis of non-infectious etiology that was not satisfactorily controlled by either corticosteroid or cytotoxic agent therapy. Lymphocyte cultures are prepared where the immune cells are tested against various crude ocular extracts, as well as purified human S-antigen, to assess evidence of cellular immune memory, which is considered to be the in vitro equivalent of the anamnestic response in vivo. Patients chosen will be treated with CsA. During this period, the patients' clinical, immunologic, and ocular electrophysiologic course will be closely monitored. Specific attention will be given to renal function changes since this is a frequent side effect. In the second arm of this study, patients with active inflammatory disease but not on therapy will be randomized to either corticosteroids or cyclosporine. Patients between the age of 12 to 60 years are eligible. This study is double-masked.

Major Findings: CsA has been found to be effective in the treatment of some cases of posterior uveitis. An improvement in the inflammatory activity and visual acuity was seen in most patients treated to date. Patients with the ocular manifestations of Behcet's disease appear to be particularly responsive to this agent. The improvement in the clinical condition could be supported by a concomitant improvement in electrophysiologic testing, particularly contrast sensitivity. Patients treated with CsA had no abnormalities of natural killer cell activity before the initiation of therapy, nor was any noted after. The OKT4/OKT8 ratio fell during CsA therapy, reflecting an increase in the OKT8 fraction. CsA significantly decreased skin test responsiveness but did not alter lymphocyte proliferation or antibody production. CsA would appear to be an effective therapeutic mode for well-defined cases of sight-threatening posterior uveitis, but definitive answers should come with the results of the randomized trial.

Significance to Biomedical Research and the Program of the Institute: Uveitis is one of the most frustrating problems in all of ophthalmology. Present modes of therapy for patients with severe ocular inflammatory disease are

inadequate, non-specific, and have myraid side effects. CsA appears to be effective in treating posterior uveitis of noninfectious etiology. This is the first new agent in decades to be found to be useful in the severe form of this condition. Additionally, because of CsA's unique activity against only a subset of T-cells, the possible therapeutic response underscores the apparently mandatory role of the T-cell in this disorder.

Proposed Course: Studies of cyclosporine therapy in uveitis will continue, with the recruitment of patients particularly for the randomized trial.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Nussenblatt RB, Palestine AG, Rook AH, and Scher I: Cyclosporine therapy for intraocular inflammatory disease. Lancet ii:235, 1983.

Nussenblatt RB, Palestine AG, and Chan CC: Cyclosporine therapy in the treatment of intra-ocular disease resistant to systemic corticosteroids or cytotoxic agents. Am J Ophthalmol 96:275, 1983.

Nussenblatt RB, Palestine AG, Chan CC, Leake WB, Rook AH, and Scher I: Cyclosporine therapy in the treatment of uveitis. Transplantation Proc XV (Suppl 1):2914, 1983.

Nussenblatt RB: Treatment of autoimmune uveitis. In Current Therapy in Allergy and Immunology 1983-1984, Lichtenstein LM, Fauci AS, editors. Philadelphia, Pennsylvania, BC Decker Inc, 1983, pp 224-227.

Gery I, Nussenblatt RB: Immunosuppressive drugs and therapy in ophthalmology. In Pharmacology of the Eye, Rose NR, Mackay IR, editors. Berlin, Springer-Verlag (in press).

Nussenblatt RB, Palestine AG, Chan CC, Breen L, and Caruso R: Improvement of uveitis and optic nerve disease by cyclosporine in a patient with multiple sclerosis. Am J Ophthalmol (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00116-04 CB

PERIOD COVERED
October 1, 1983 to september 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Double-Masked Treatment of Ocular Toxoplasmosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Francisco deMonasterio	M.D., D.Sc.	Head, Section on Visual Processing	CB, NEI
	Daniel Seigel	D.Sc.	Deputy Chief	OBE, NEI
	Marvin Podgor	M.S.	Statistician	OBE, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the project is to evaluate whether clindamycin combined with sulfadiazine will prove as or more effective in the treatment of ocular toxoplasmosis than the combination of sulfadiazine and daraprim. Patients with active toxoplasmosis will be randomized within strata (determined by size of lesion and proximity to the macula) to one of the two treatments in this double-masked study.

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 81-EI-92

Objectives: Ocular toxoplasmosis represents a sizeable number of cases seen in a uveitis clinic. Sulfadiazine and daraprim have been considered the combination of choice in the therapy of sight-threatening toxoplasma lesions. Reports have now suggested that clindamycin may be effective therapy for toxoplasmosis. The objective of this study is to randomize patients in a double-masked study to compare the efficacy of sulfadiazine/clindamycin and sulfadiazine/daraprim therapy.

Methods Employed: Patients 18 years or older, of either sex (females not pregnant), are being admitted to this study. They must manifest an active retinal lesion due to toxoplasmosis. Patients receive a standard ophthalmic examination and are randomized into a therapy group on the basis of the size and position of the active lesion. The cause of the disease will be followed clinically, as well as with electrophysiologic testing. The data is to be collected by the NEI Office of Biometry and Epidemiology and evaluated.

Major Findings: This study does not yet have enough participants for there to be any significant findings.

Significance to Biomedical Research and the Program of the Institute: Toxoplasmosis is the cause of a large number of uveitis cases in the United States. Daraprim has potentially serious side effects. If another form of therapy can be demonstrated equally or more effective than sulfadiazine and daraprim, then clinicians will be given an expanded choice in dealing with this potentially sight-threatening problem.

Proposed Course: Studies on double-masked treatment of ocular toxoplasmosis will be discontinued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 EY 00159-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Cyclosporine Therapy of Childhood Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
	William Leake	M.S.	Biologist	CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efficacy of cyclosporine, an endecepeptide with specific anti-T-cell characteristics, is being tested in children with sight-threatening ocular inflammatory disease of non-infectious origin who have failed on conventional therapy.

GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 83-EI-95

Objectives: We have found that cyclosporine is effective in treating sight-threatening uveitis in adults who previously had a negative therapeutic response with corticosteroids and/or cytotoxic agents. Many of the diseases treated effectively with cyclosporine in adults occur in children as well. The goal of this study is to investigate whether cyclosporine is effective in treating sight-threatening posterior uveitis of non-infectious etiology in children.

Methods Employed: Children, aged 12-18 years, will be selected for this study. All patients will have bilateral sight-threatening uveitis of non-infectious etiology. The cellular immune response to the retinal S-antigen of these patients will be evaluated, though this is not a criteria for entry into the study. Patients will be treated with cyclosporine alone initially, during which their clinical, immunologic, and electrophysiologic course will be closely monitored.

Major Findings: The initial results appear to be promising, particularly in the treatment of patients with pars planitis. However, a longer followup period is needed.

Significance to Biomedical Research and the Program of the Institute: Uveitis is one of the most frustrating problems in all of ophthalmology, and this is particularly the case when it occurs in children. The difficulties in treating children with uveitis are enormous, and the potential side effects are a matter of special concern. It will be of great import if cyclosporine proves to be effective in this age group. It will provide the treating physician with a new modality which will possibly have fewer side effects.

Proposed Course: This study will continue in order to enroll the number of patients needed and to follow those on therapy for a longer period of time.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Palestine AG, Nussenblatt RB, and Chan CC: Cyclosporine therapy of uveitis in children. In Excerpta Medica International Congress Series, Patel S, editor. Amsterdam, Netherlands, Elsevier Science Publishers BV (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00158-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Treatment of Cicatricial Pemphigoid Utilizing Cyclosporine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
	William Leake	M.S.	Biologist	CB, NEI

COOPERATING UNITS *(if any)* Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Boston, Massachusetts (C. Stephen Foster, M.D.); National Cancer Institute, Dermatology Branch (Steve Katz, M.D.); Bascom Palmer Eye Institute, Miami, Florida (William Culbertson, M.D.)

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cyclosporine is a drug with specific anti-T-cell actions. Cicatricial ocular pemphigoid is a progressive disease of the conjunctiva of the eye which causes dryness, trichiasis, and ocular scarring leading to blindness. The conventional therapy for this is the use of azathioprine or cyclophosphamide in combination with systemic corticosteroids. Ocular cicatricial pemphigoid has, in general, been thought to be an antibody-mediated disease. However recent evidence has demonstrated that biopsies of the inflamed conjunctiva have an elevated T-cell helper-to-suppressor ratio. Since cyclosporine has specific anti-T-cell activities and may be less toxic than the conventional therapy, this drug will be administered to patients with ocular cacatricial pemphigoid to test its efficacy in the treatment of this disease.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 83-EI-32

Objectives: Cyclosporine has been shown to have significant and specific anti-T-cell activity in a variety of animal and human diseases. It has been observed that the abnormal T-cell helper-to-suppressor ratio in biopsy specimens from patients with cicatricial pemphigoid will reverse when these patients are treated with conventional immunosuppressive therapy. The goal of this study will be to test the efficacy of cyclosporine in treating patients with ocular cicatricial pemphigoid.

Methods Employed: Patients age 18 and older who have cicatricial pemphigoid, preferably with ocular involvement, will be admitted to this study. They may or may not have other organs involved with cicatricial pemphigoid. Pretreatment conjunctival biopsies will be done for T-cell typing and immunofluorescence. Only patients with positive immunofluorescence on conjunctival biopsy at the basement membrane zone will be considered for inclusion. After patients are treated for three months with systemic cyclosporine, conjunctival biopsy will be repeated. If the patient has improved, the therapy will be continued. If the patient's condition has worsened during this three month period, the therapy will be discontinued and conventional therapy will be instituted. During the period of therapy, the patient's clinical and immunologic course will be closely monitored.

Major Findings: This study is still recruiting patients. The initial clinical results require a longer follow-up.

Significance to Biomedical Research and the Program of the Institute: Cicatricial pemphigoid is a severe sight-threatening condition which is slowly progressive. The conventional therapy, cytotoxic agents in combination with prednisone for long periods of time, is often effective but is accompanied by significant side effects. Cyclosporine may be an important alternative in therapy since it may have fewer severe long-term side effects as compared to cytotoxic agents. Furthermore, because cyclosporine is a specific anti-T-cell drug, its effectiveness in this autoimmune disease will further support the evidence that cell-mediated immunity is an important pathogenetic mechanism in ocular cicatricial pemphigoid.

Proposed Course: We will continue to recruit patients with cicatricial pemphigoid for the long-term effects, treatment with cyclosporine, and follow patients to evaluate.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Disease (Other Infections)

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00185-01 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
The Treatment of Retinitis Pigmentosa With Cyclosporine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
Others:	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI
	Muriel I. Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI

COOPERATING UNITS *(if any)*

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Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ten retinitis pigmentosa patients showing in vitro proliferative responses to the retinal S-Ag will be entered into this randomized double-masked study. Patients will receive either cyclosporine or placebo. Patients will be followed for at least one year.

Project Description:

Other Professional Personnel Engaged on Project:

Rafael C. Caruso	M.D.	Expert	CB, NEI

Protocol Number: 83-EI-185

Objectives: To determine whether T-cell mediated disease plays a significant role in the retinal alterations occurring in retinitis pigmentosa patients who demonstrate positive in vitro responses to the retinal S-Ag.

Methods Employed: Retinitis pigmentosa patients with good central vision who "respond" in vitro to the S-Ag will be randomized to either cyclosporine or placebo therapy. Electrophysiologic testing, visual acuities, and photos will be obtained by masked observers. After one year, the clinical and physiologic data will be compared between the two groups.

Major Findings: The study has just begun and results are forthcoming.

Significance to Biomedical Research and the Program of the Institute: Retinitis pigmentosa has no known therapy. Recent evidence might suggest that a subgroup of these patients potentially have an immune component to their disease. If they can be successfully treated, this would be the first therapy for this disorder.

Proposed Course: Patients will continue to be recruited, and those randomized will be followed.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00184-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
T-Cell Lines and Clones in Uveitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rachel Caspi	Ph.D.	Visiting Fellow	CB, NEI
Others:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
	Magda El-Saied	M.B.	Visiting Associate	CB, NEI
	Consuelo Muellenberg-Coulombre		Chemist	CB, NEI
	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI

COOPERATING UNITS *(if any)*

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Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.07	1.07	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Long-term T-cell lines and T-cell clones are maintained in vitro from both peripheral blood and ocular fluids of humans and animals. The goal of these studies will be to identify the immunoreactive cells and mediators involved in the intraocular inflammatory process.

Project Description:

Other Professional Personnel Engaged on Project:

Sanford Meyers M.D. Senior Staff Ophthalmologist CB, NEI

Protocol Number: 82-EI-169

Objectives: Long-term maintenance of T-cells in vitro permits the investigators to preferentially separate and grow various T-cell subsets in vitro. The goal is to produce various T-cell lines and clones from lower mammals and humans having ocular inflammatory disease. This will permit the identification of clones capable of ocular immunomodulation. The understanding of these mechanisms will help not only in the development of new therapies but possibly in prevention.

Methods Employed: Lewis rats and other lower mammals are immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad. After the development of disease, cells from the blood, lymph nodes, or eyes are taken, washed, and prepared. For humans, lymphocytes are taken either from the peripheral blood or from the eye as a consequence of a clinically indicated vitrectomy. Cells obtained are placed in microtiter culture plates and stimulated either with mitogen or S-antigen. After this initial clonal expansion, long-term cell lines and clones are developed either by the use of the double-agar or limiting dilution technique. These clones or lines are then tested for functional capacities, mediators, and cell surface characteristics.

Major Findings: S-antigen specific T-cell clones can be obtained from peripheral blood, lymph node, spleen, and ocular fluids. Cells from intraocular sources may not always express T-cell surface markers that are identifiable with commercially obtained antisera, but both $OKT8^+$ and $OKT4^+$ identifiable have been obtained. Long-term cell lines from the rat are capable of inducing ocular disease when given to a naive host, while early indications suggest that in some situations a suppression of the disorder may be obtained.

Significance to Biomedical Research and the Program of the Institute: It has become increasingly clear that the T-cell plays an important role in many types of intraocular inflammatory conditions. Cloning technology permits the study of the cell(s) involved in this response, and the identification of these cells will open new vistas to our understanding.

Proposed Course: This project will continue so that a battery of clones will be identified.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Rozenszajn LA, Radnay J, Nussenblatt RB, and Sredni B: Human lymphoid cells and their progenitors--isolation, identification and colony growth. In Hematopoiesis (Methods in Hematology series) (in press).

Nussenblatt RB, Palestine AG, El-Saied M, Meyers S, Lando Z, Mullenberg C, and Rozenszajn LA: Long-term antigen specific and non-specific T-cell lines and clones in uveitis. Current Eye Research 3:299, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00133-01 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Virologic and Immunopathologic Aspects of Eye Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John J. Hooks	Ph.D.	Microbiologist	CB, NEI
Others:	Caroline Percopo	A.B.	Biologist	CB, NEI
	Muriel Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	
	Barbara Detrick-Hooks	Ph.D.	Expert	CB, NEI

COOPERATING UNITS *(if any)*

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SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initiated studies to evaluate the possible involvement of viruses in the etiology and pathogenesis of a variety of eye diseases such as retinal degeneration and uveitis. We have also continued our studies on lymphokines and regulatory cells in retinal degenerative disorders. Retinitis pigmentosa is a group of diseases of unknown etiology which is characterized by degeneration of the retina and the associated retinal pigment epithelial cell. The pathophysiologic processes involved in the maintenance of retinal integrity is only partly understood and the processes involved in retinal degenerations are not known. We have identified an abnormality in a regulatory protein (interferon-gamma) and an altered expression of a cell surface antigen (HLA-DR) in this disease. These data point to the possibility of an underlying abnormality in biologic control mechanisms in retinitis pigmentosa.

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 83-EI-185

Objectives: To determine whether patients with retinal degenerative disorders have a defect in biologic regulatory control systems.

Methods Employed: Peripheral mononuclear cells were obtained from Ficoll-Hypaque separated heparinized blood. HLA-DR antigen bearing cells were identified by the lysis of cells with anti-DR antibody and complement.

Interferon-gamma was prepared by incubating peripheral mononuclear cells (2.5×10^6 cells/ml) with conconavalin A (Con A-10 ug) or phytohemagglutinin (PHA-4ug) for 48 hrs at $37^{\circ}C$. Supernatant fluids were assayed for antiviral activity. Antiviral activity was determined by the reduction in vesicular stomatitis virus plaque formation on human amnion (WISH) cells grown in microtiter plates.

Major Findings: Peripheral lymphocytes from patients with retinitis pigmentosa have a deficiency in their ability to produce the lymphokine interferon-gamma. In contrast, cells from these patients produce normal amounts of interferon-alpha in response to viruses. Moreover, the patient's white blood cell amount, platelet count, differential count, and serum immuglobulin levels were all within normal ranges.

We found that monocytes from retinitis pigmentosa patients express diminished amounts of class II (HLA-DR) antigens in comparison to normal individuals, normal siblings of retinitis pigmentosa patients, glaucoma patients and macular degeneration patients. This observation is correlated with a subnormal production of IFN-gamma, a potent regulator of class II antigen expression. When monocytes from retinitis pigmentosa patients are treated with IFN-gamma, the decreased expression of DR on the cell surface is restored to levels found on monocytes from normal individuals.

Significance: RP is a disease affecting over 400,000 people in the USA and over one million people throughout the world. The pathophysiologic processes involved in the maintenance of retinal integrity is only partly understood and the processes involved in retinal degeneration are not known. The demonstration of an imbalance in systemic regulatory control systems raises the possibility that these or related regulatory proteins and cell surface receptors may be instrumental in maintaining retinal integrity. A better understanding of the underlying defects in retinal degenerations may provide a rational for attempts to manage or limit the progression of this disease.

Proposed Course: This study will continue to evaluate the imbalance in systemic regulatory control systems which we have identified in patients with retinitis pigmentosa. Since our preliminary studies show that the HLA-DR antigen is present on the surface of the retinal pigment epithelial cell, we will investigate the modulation and functional activities of this important ocular regulatory cell.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Hooks JJ, Detrick-Hooks B, Gris S, and Newsome DA: Retinitis pigmentosa associated with a defect in the production of interferon-gamma. Am J Ophthalmol 96:755, 1983.

Hooks JJ, Hill MR, and Detrick-Hooks B: Interferon determinations in evaluating the immune response. In Chemical Regulation of Immunity in Veterinary Medicine, Kende and Gainer, editors. New York, New York, Alan R Lirs, Inc., 1984, pp. 117-125.

Hooks JJ and Detrick-Hooks B: Interferon in autoimmune diseases and other immunoregulatory disorders. In Interferon and the Immune System, Vilcek E and DeMaeyer E, editors. New York, New York, Acad Press (in press).

Nagai H, Sztgein MB, Steeg PS, Hooks JJ, Steinberg AD, and Openheim JJ: Diminished peripheral blood monocyte DR antigen expression in systemic lupus erythematosus. Clin & Exp Rheum (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00156-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Immune Complexes in Arthus-Type Panophthalmitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Alan G. Palestine	M.D.	Staff Ophthalmologist	CB, NEI
Others:	Robert B. Nussenblatt	M.D.	Head, Section on Clinical Ophthalmic Immunology	CB, NEI
	Chi-Chao Chan	M.D.	Staff Ophthalmologist	CB, NEI
	Paul Russel	Ph.D.	Research Chemist	LVR, NEI
	Igal Gery	Ph.D.	Visiting Scientist	LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Arthus-type panophthalmitis is an acute intraocular inflammatory condition which is produced in rats by injury of the lens following hyperimmunization using soluble rabbit lens crystallins. The onset of inflammation is rapid and the eyes progress to phthisis due to a severe granulomatous panophthalmitis in one week. This entity has been hypothesized to be a disease which is produced by circulating immune complex deposition. However, circulating immune complexes have never been measured in this experimental model. This project has been directed at the evaluation of humoral and cellular immunity in Arthus-type panophthalmitis to provide a better understanding of the general events of intraocular inflammatory disease. We determined that rats that develop this intraocular inflammation have no cell-mediated immunity to rat lens protein and have little to no cell-mediated responses to rabbit lens protein. However, they have markedly elevated titers of anti-rabbit lens crystallins. Elevated levels of circulating immune complexes were not detected in control animals but were detected in many animals that developed Arthus-type panophthalmitis. However, these circulating complexes were also detected in animals prior to lens injury when there was no intraocular inflammation and also in animals immunized with bovine serum albumin. Deposition of IgG and C3 was detected in the eyes of animals with Arthus-type panophthalmitis.

Project Description:

Other Professional Personnel Engaged on Project: None

Objectives: The objective of this project is to understand the mechanisms of intraocular inflammatory disease (uveitis). Since there are drugs available which are able to act on specific sites in the immune system, an understanding of the role of cell-mediated and humoral-mediated immunity in the production of uveitis is essential.

Methods Employed: C1Q precipitation and Raji cell assays for immune complexes were utilized. Lymphocyte stimulation assays to lens protein, as evidenced by tritiated thymidine incorporation, were used to assess cell-mediated immunity. Immunofluorescent techniques on frozen tissue were used to assess tissue deposition of immunoglobulin and complement. The ELISA method for quantitating anti-crystallin antibody titers was also used.

Major Findings:

1. There is little evidence for cell-mediated immunity to rat lens protein in animals immunized with rabbit lens extract. This implies that in the initial stages of Arthus-type panophthalmitis, cell-mediated immunity probably plays a minor role.

2. Immunization with rat lens crystallins results in high titers of alpha, beta, and gamma anti-crystallins.

3. Although circulating immune complexes were found in some animals that developed Arthus-type panophthalmitis, they were also found in animals prior to lens injury when there was no intraocular inflammation and in animals immunized with bovine serum albumin. This implies that, although circulating immune complexes may be detected in diseases, one cannot necessarily infer that they are pathogenic. Specifically, the detection of circulating immune complexes does not prove that deposition of these immune complexes is responsible for the observed pathology.

4. There was deposition of IgG and C3 in the anterior segment of the inflammed eyes. This, in combination with the elevated anti-crystallin serum titers and the lack of correlation with circulating immune complexes, implies that the early stages of this inflammatory Arthus-type panophthalmitis are mediated by antibody deposition and complement fixation to lens protein released from the injured lens. We did not detect deposition of C3 and IgG in the choroid. However, the choroid becomes involved with a diffuse granulomatous inflammation, eventually extending to all structures of the eye. This occurs beginning four days after lens injury. It is possible that this inflammation is secondary to severe alterations of the ocular structures from the ongoing anterior inflammation due to antibody-antigen interaction.

Project No. ZO1 EY 00156-02 CB

Significance to Biomedical Research and the Program of the Institute: Intraocular inflammation (uveitis) is a significant cause of visual handicap. The mechanisms of the production of the various types of clinically observed uveitis are not well understood. Arthus-type panophthalmitis is a severe inflammation which in some aspects mimics human phaco-anaphylactic endophthalmitis. However, the primary importance of this work is the demonstration that some types of intraocular inflammation can be produced using autologous antigen as the intraocular stimulus without the participation of cell-mediated immunity. Because drugs such as cyclosporine which are specific for suppression of only certain parts of the immune system are now available, a better understanding of the participation of the various arms of the immune system is important in determining the role of immunosuppressive agents in the treatment of intraocular inflammatory disease.

Proposed Course: An attempt is being made to induce Arthus-type panophthalmitis using a monoclonal antibody to beta-crystallin. This would further demonstrate that humoral-mediated immunity can produce this experimental model.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Palestine AG, Nussenblatt RB, Chan CC, Russell P, and Gery I: The role of immune complexes in Arthus-type panophthalmitis in the rat. Invest Ophthalmol Vis Sci (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00157-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Uveitis and Immune Complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Alan G. Palestine M.D. Staff Ophthalmologist CB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Branch

SECTION
Section on Ophthalmic Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Circulating immune complexes have been demonstrated in some patients with autoimmune uveitis. In addition, immune complexes have been demonstrated in the anterior chamber in patients with active inflammation. However, there is no clear understanding of the role of these immune complexes in the production of the inflammation. Specifically, it has not been proved that the presence of immune complexes is evidence that they are participating in the intraocular inflammation. This study is directed at attempting to investigate this question by studying both intraocular and circulating immune complexes and complement activation.

Project No. ZO1 EY 00157-02 CB

Project Description:

Other Professional Personnel Engaged on Project: None

Protocol Number: 82-EI-159

Objectives: The objective of this project is to understand the role of immune complexes in the production of intraocular inflammation.

Methods Employed: Methods employed include analysis of immune complexes by C1Q and Raji cell assays and analysis of activated fragments of complement using a radio-immune assay.

Major Findings: This study is still recruiting patients and no findings can be presented at this time.

Significance to Biomedical Research and the Program of the Institute: It has generally been assumed that the presence of immune complexes in the serum aqueous implies that these are involved in the pathogenesis of a particular inflammatory disease. However, recent evidence has shown that they may not be involved in the inflammatory process, and it has been suggested that in some cases circulating immune complexes may be protecting rather than pathogenic. Since the basic mechanisms of uveitis are poorly understood, the general approach to the treatment of uveitis has been the use of non-specific immunosuppressive agents applied locally or systemically. Analysis and determination of the actual mechanisms of intraocular inflammation would permit the development of more specific therapies.

Proposed Course: Recruitment of patients for this study will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Infammatory Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00065-07 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophysiological Studies of the Visual System of Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Francisco de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
Others:	Stanley J. Schein	Ph.D.	Expert	CB, NEI
	Edna P. McCrane	B.S.	Biologist	CB, NEI
	Andrew Mariani	Ph.D.	Staff Fellow	LVR, NEI

COOPERATING UNITS *(if any)*

Laboratory of Neuropsychology, NIMH (R. Desimone, J. Moran)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	0.65	0.35

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves the study of the physiological organization of neurons in the visual system of non human primates. The project gives emphasis to the chromatic and spatial properties and central projections of neurons of the retina, lateral geniculate body, striate cortex, and extrastriate cortex of macaque monkeys.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To study the neural organization underlying the processing of visual information at different levels of the visual system.

Methods Employed: Intracellular and extracellular recordings from single neurons, and extracellular recordings of mass responses.

Major Findings:

1. Area V4 is a visuotopically organized region of macaque extrastriate cortex that receives a major input from area V2 and is a major source of visual input to inferior temporal cortex. Single neurons were studied within the representation of the central 5^{o} of V4 in anesthetized and paralyzed macaques, with a semi-chronic preparation. The cells could be divided into two groups based on their responses to gratings. Both groups had receptive fields of the same width, equal to 0.9^{o} + 4 x eccentricity. The optimal spatial frequency for cells of the first group had a period greater than the receptive-field width, while that of cells of the second group had a period equal to or smaller than the width of the receptive field. Thus, the first group seems to show summation within the field, while the second group seems to show interactions within the field. Some (but not all) of the properties of the two groups appear to be analogous to properties of striate simple and complex cells, respectively. Cells of both groups normally had a large suppressive zone located around the excitatory receptive field, extending up to $10\text{-}20^{o}$ from the center of the activating center. Although dark bars, light bars, annuli, and gratings did not elicit any response from the suppressive zone alone, such stimuli often reduced the response to a simultaneous, non-overlapping, central stimulus by 80% or more.

2. Like other extrastriate areas, V4 contains a representation of the contralateral visual field. Within the central 5^{o}, the excitatory, central activating area of V4 receptive fields rarely extends more than 1^{o} across the vertical meridian into the ipsilateral visual field. The suppressive surround of these cells, however, extends far into the ipsilateral field. In recordings from anesthetized and paralyzed macaques, receptive-field centers were all in the contralateral receptive field, the mean overlap on the vertical meridian being of only 0.6^{o}. The suppressive surround was tested by the paired presentation of central stimulus and a large annulus; the elicited responses were compared to those elicited by the central stimulus alone. The mean suppression across all cells was 75%, the responses of 74% of the cells being significantly suppressed by the annulus. These results were corroborated in one alert, behaving macaque. The extent of the suppressive surround into the ipsilateral field was measured by comparing the responses to a central stimulus to those elicited by a central stimulus paired with a bar confined to the ipsilateral field at different locations from 0^{o} to 16^{o} from the vertical meridian. Across all cells suppression was significant out to at least 16^{o}, ranging from 58% at 0^{o} to 18% at 16^{o}. V4 receives a heavy commissural projection not strictly limited to the vertical meridian representation. To determine the contribution of the corpus callosum to the ipsi-

lateral suppression, the posterior half of this commissure was sectioned in one of the macaques. Whereas the suppression by an annulus remained equal to that in normal animals, the suppressive effect of an ipsilaterally located bar was significantly reduced. Across all animals suppression was significant only out to 4^{o} from the vertical meridian; even at 4^{o} the suppression was only half of that seen in the normal animal.

3. The effects of steady and of flashed backgrounds were examined on V4 cell responses. The background lights were large fields of low retinal illuminance. The cells responded well to white light and lacked color-opponent responses. In some cells the summed responses could be equalized by CIE-equiluminous stimuli, while other cells exhibited obvious departure from response equalization; the present experiments were restricted to the latter cells. Steadily presented chromatic backgrounds significantly modified the spectral bias of the cells in a manner consistent with an adjustment of the balance of different afferent cone signals to the amount and quality of the prevailing illumination, in a manner consistent with von Kries' Coefficient Law. Different backgrounds changed the spectral bias of the cells. The bias depended on the adaptation history of the neuron, and it was not an invariant property. Chromatic backgronds simultaneously flashed with lights of different wavelength produced a spectrally differential, transient desensitization of cell firing at background onset and offset. Response desensitization could be obtained with backgrounds of a color similar to or different from that of the test lights, respectively indicating homochromatic and heterochromatic masking. The phenomena can be ascribed to antecedent visual levels, as similar desensitizations can be evoked in the responses of retinal ganglion cells. The V4 desensitization could induce "green/magenta" or "blue/yellow" opponent-like responses. Unlike typical color-opponent behavior, however, such induced responses showed the same changes in firing at both stimulus onset and offset, i.e., on-excitation and off-excitation to some wavelengths, and on-inhibition and off-inhibition to others. Because in some conditions the induced behavior might mimic a double color-opponent behavior, the results are relevant to spectral classifications of V4 cells based on surface-mode or aperture-mode color stimuli.

4. A physiological study of "blue-yellow" opponent ganglion cells was carried out with emphasis on their spatial properties. Two types of "blue-center, yellow-surround" cells have been found, one with a small receptive-field center similar to that of the more commonly observed "red-" and "green-center" cells, and another with a larger receptive-field center similar to that of "yellow-center" cells. These two varieties have different retinal distributions. Small field cells were more common than large field ones in the foveal region, while the reverse was found in the examined extent of the extrafoveal region. Detailed mappings of the receptive field, based on sensitivity profiles taken along a grid of parallel locations covering the field, indicate that small field "blue-center" cells occasionally show an additional, single, secondary peak of sensitivity whose distance from the main central peak depends on eccentricity. This finding is consistent with recent anatomical findings reported by Mariani on the contacts between blue-sensitive cones and bipolar cells.

5. The correlation between receptive field and dendritic field sizes of ganglion cells of macaque retina was examined using single-cell recordings and Golgi-impregnation techniques. The results indicate a very close correlation between these two types of measure at different retinal eccentricities. The results were also analyzed in terms of the anatomical and physiological characteristics of the so-called X/beta, Y/alpha, and W/gamma ganglion cells. A most important result has been the finding of two physiological types of X-like cells and of two anatomical types of beta-like cells. The field sizes of these two X/beta-like types are similar to one another in the foveal region, but become easily distinguishable in more peripheral retina. Anatomically, one of these types corresponds to Polyak's midget ganglion cells, while the other resembles his Parasol cells; physiologically, the former type are color-opponent cells with overt surround antagonism, while the latter type are color-opponent cells with concealed surround antagonism. This finding indicates a significant functional difference between the retinal organization of felines and primates in terms of ganglion cell varieties.

Significance to Biomedical Research and the Program of the Institute: Understanding the organization of the visual system of non-human primates is most valuable for understanding the mechanisms of visual processing of the human visual system.

Proposed Course: These studies will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Structure and Function--Cell and Systems)

Publications:

de Monasterio FM: Electrophysiology of Color Vision: Cellular level. Doc Ophthalmol Proc Series 69:9, 1984.

de Monasterio FM: Color selectivity of V4 and induced spectral properties. Neurosci Abstr 9:152, 1983.

de Monasterio FM: Effects of steady and flashed backgrond lights on the responses of macaque V4 cells. J Neurophysiol (in press).

Desimone R and Schein SJ: Receptive field properties of neurons in visual area V4 of the macaque. Neurosci Abstr 9:153, 1983.

Moran J, Desimone R, Schein SJ, and Mishkin M: Suppression from ipsilateral visual field in area V4 of the macaque. Neurosci Abstr 9:240, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00059-06 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrophysiological and Psychophysical Evaluation of Retinal Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
Others:	Rafael C. Caruso	M.D.	Expert	CB, NEI
	Kent E. Higgins	Ph.D.	Expert	CB, NEI
	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB, NEI
	Myles J. Jaffe	O.D.	Staff Fellow	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Branch

SECTION
Section on Visual Processing

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.93	0.85	2.08

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a general support- and service-providing project for the study and collaborative diagnosis of inflammatory, degenerative, or congenital disorders, and to conduct research that can be applied to the development of electrophysiological and psychophysical procedures for measuring visual function parameters in patients of NEI's Eye Clinic and of other clinical care services in the NIH Clinical Center.

Project Description:

Additional Personnel Engaged on Project:

Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI
Doris J. Collie	A.A.	Health Technician	CB, NEI
Mary E. Fuhrman		Health Technician	CB, NEI
Jacqueline D. Robinson		Secretary, SVP	CB, NEI

Protocol Number: None

Objectives: To improve diagnosis or evaluation of visual function in toxic, inflammatory, degenerative, and congenital visual disorders affecting the retina and visual pathways. To develop clinical procedures for the study of visual function in the clinical setting.

Methods Employed: We use commercially available and laboratory-developed instruments for the measurment of visual function parameters in both normal volunteers and NIH patients. Tests involve electroretinography, visually evoked potentials, electro-oculography, spatial contrast sensitivity, sensory rod and cone thresholds, color vision testing, retinal image stabilization, visual perimetry, and other psychophysical and electrophysiological measurements.

Major Findings: Two types of activities are covered under this project. One includes service-providing studies of visual function for the supportive or collaborative diagnosis, evaluation, and follow-up of patients with inflammatory, degenerative, toxic, or congenital disorders. These activities include all of the routine electrophysiological and psychophysical testing of inpatients, outpatients, and referred consult cases seen in the NEI's Eye Clinic. In this fiscal year about 2300 tests will be performed, with a total of ca. 14,000 tests performed over the last six years. In addition, these activities also provide most of the current "specialized" testing on visual sensory neural function of patients, often under collaborative arrangements with other sections of the Clinical Branch.

The other activity involves the development of new tests for clinical studies using non-invasive electrophysiological and psychophysical measurements. Such tests, developed under this project, are, upon successful completion, assigned to separate projects for normative and clinically applied studies. Efforts continue toward the development of tests centered around a system of retinal-image stabilization which is currently being used for studies of selected cases.

The nature of these activities is such that no publications result directly from this project, as successful work or techniques are included

in or transferred to more specialized projects or studies. Nevertheless, much of the patient-test results published under other projects of the Clinical Branch of the NEI have been obtained, even if initially, under the present project.

Significance to Biomedical Research and the Program of the Insitute: This project provides all of the routine electrophysiological and psychophysical testing of patients of the NEI's Eye Clinic in terms of visual sensory neural function. Development of new research techniques and the application of new and existing tests to clinical studies help in the diagnosis and management of visual disorders and in the understanding of physiopathological mechanisms of retinal disease.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Noninvasive Techniques in the Study of Retinal Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00122-04 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Anatomical Studies of the Visual System of Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
Others:	Stanley J. Schein	Ph.D.	Expert	CB, NEI
	Edna P. McCrane	B.S.	Biologist	CB, NEI
	Andrew Mariani	Ph.D.	Staff Fellow	LVR, NEI
	J. K. Newlander		Summer Student	CB, NEI

COOPERATING UNITS *(if any)*

David A. Kostic Normal Volunteer CC, NIH

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.96	0.35	0.61

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The blue-sensitive cones of the macaque retina are selectively labelled by tissue-reactive dyes injected into the vitreous humour. We have examined the dependence of blue cone density on retinal eccentricity along the temporal and nasal segments of the horizontal meridian. We have also studied a selective staining of some postreceptoral cells. These studies provide information of the probable retinal circuitry of the blue-sensitive cone pathway of primate retina.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To study the anatomical properties and neural organization of the primate visual system.

Methods Employed: Histological processing of retina, including whole mounts; intravitreal injection of tissue-reactive dyes; computer modelling and statistical analyses of point patterns; electron microscopy; silver cell and myelin stain; and histological processing of cerebral cortex.

Major Findings:

1. Intravitreal injection of some fluorescent and non-fluorescent tissue-reactive dyes results in the selective, intracellular staining of a specific population of cones of macaque retina that can be indirectly identified as blue-sensitive cones because of their retinal distribution, angular separation and predicted visual resolution, susceptibility to retinal insult, and differential incidence in different mammalian species. We have quantitatively examined density profiles of the blue cones as a function of retinal eccentricity, from 2^{o} to about 60^{o} along the nasal and temporal segments of the horizontal meridian of the retina of rhesus monkeys. In the extrafoveolar retina, 2^{o} to 60^{o} eccentricity, different model functions were fitted to the decay of density with eccentricity of these profiles. This decay is well described by the sum of two single exponential functions, indicating that the decline of blue cone density is best described by an exponential decay controlled by two factors: One regulates the density decay over the entire extrafoveolar range, while the other co-regulates the decay over the central part of this range, with a 1/e space constant of ca. 5^{o} temporally and 6^{o} nasally. The square root of the reciprocal of the former factor is roughly similar to the normalized averaged separation between blue cones, whereas the latter factor seems to have a reciprocal relationship with rod density. It is inferred that blue-cone mediated visual resolution follows an exponential decay with eccentricity, a conclusion that agrees with the exponential decay of (all-cone) human visual acuity from central to peripheral retina.

2. In the foveolar retina, 0^{o} to 2^{o} eccentricity, the density of blue-sensitive cones declines significantly, reaching a local minimum at the center of the foveola. The blue cones are rare in a (square) central foveolar region of ca. 0.3^{o} on the side, and they are not found in the (square) central-most region of 0.15^{o} on the side. The latter region is slightly smaller than the so-called bouquet of central cones, and smaller than the rod-free area. The findings support the psychophysical observations that the physiological "small-field" tritanopia of the normal human central fovea is indeed due to a scarcity of blue cones.

3. The mean separation between blue cones in the parafoveolar region of their peak density is of $3.83 \pm$ s.d. 0.56 arc-minutes (corresponding to a nominal Nyquist frequency of 7.83 ± 1.25 cycle/arc-degree for the case of a sufficiently regular array). This acuity is within the range, and agrees well

with the mean value, of blue-cone mediated acuity measured with different methods in both normal subjects and blue-cone monochromats, who show an acuity of 4.01 ± 1.69 arc-minutes (predicting a nominal Nyquist frequency of 7.5 cycle/arc-degree). The visual resolution of the blue cones, albeit significantly smaller than that of the other cone types, is quite adequate for the optical properties of the eye. The primate eye has chromatic aberration; because refractive power varies with wavelength, long wavelengths being refracted more than short wavelengths, the eye is generally myopic for blue light. The wavelength being focused on the eye, however, depends on accommodative state, shifting from longer to shorter ones as accomodation becomes active, i.e., when the target moves from the far point to the near point. Measurements of this mechanism show that, when the stimulus to accomodation is nil, the eye is focused for a wavelength of 685 nm, and it is myopic by 1.5 D at 450 nm (a wavelength close to the peak sensitivity of blue cones at the corneal level). Such a refractive error has been found to correspond to a Snellen acuity of between 20/85 and 20/110, a value representing a nominal resolution of 6-7 cycle/arc-degree and agreeing well with both the blue-cone mediated mean acuity of humans and the mean peak-density spacing of the blue cones of simians. These results are relevant to the problem of aliasing in the visual system.

4. Two types of postreceptoral retinal cells are selectively stained following the intravitreal injection of the fluorescent dye Lucifer yellow VS in macaque retina. These are some horizontal and bipolar cells; such horizontal cells are also selectively stained by the electron-dense dye Procion black Spl. Fluorescence microscopy of Lucifer-stained retinas, electron microscopy of Procion-stained retinas, and light microscopy of Golgi-impregnated retinas show that the horizontal cells correspond to a fraction of the type I (but not type II) horizontal cells of monkey retina, and that the bipolar cells correspond to a midget-like bipolar cell whose axon terminals distribute at the level of the putative "on-center" sublayer of the inner plexiform layer. These results and their interpretation are important to the retinal circuitry of the blue-sensitive cone pathway, and provide an anatomical site for some color-opponent adaptive anomalies of this pathway found in psychophysical studies of human color vision.

Significance to Biomedical Research and the Program of the Institute: Detailed information on the anatomical properties of blue-sensitive cones was unavailable until the advent of the studies described above. These studies and the phenomemon which makes them possible, i.e. the selective labelling of blue cones, are relevant not only to the functional properties of the blue cone system investigated in different basic disciplines, but also to the clinical research and diagnosis of acquired retinal disease.

Proposed Course: These studies will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction.

Publications:

McCrane EP, de Monasterio FM, Schein SJ, and Caruso RC: Non-fluorescent dye staining of primate blue cones. Invest Ophthalmol Vis Sci 24:1449, 1983.

Shapiro MB, Schein SJ, and de Monasterio FM: Regularity and structure of the spatial pattern of blue cones in macaque retina. J Am Stat Assoc (in press).

de Monasterio FM, McCrane EP, Newlander JK, and Schein SJ: Density profile of blue-sensitive cones along the horizontal meridian of macaque retina. Invest Ophthalmol Vis Sci (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00173-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Influence of Dopamine on the Electroretinogram

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Francisco M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
Others:	Myles J. Jaffe	O.D.	Staff Fellow	CB, NEI

COOPERATING UNITS *(if any)*
Saint Elizabeth's Hospital, NIMH
Experimental Therapeutics Branch, NINCDS (G. Bruno)
Neuropsychiatry Branch, NIMH (C. Karson)

LAB/BRANCH
Clinical Branch

SECTION
Section on Visual Processing

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.30	1.30	0.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The computer-averaged Ganzfeld electroretinogram of patients with chronic schizophrenia off-medication (neuroleptics) demonstrates an increased implicit time of the cone a-wave when compared with a normal population. In addition, the first oscillatory potential has an increased implicit time. These findings suggest that both photoreceptor function and the function of cells within the outer and inner nuclear layers are sensitive to the influence of a dopamine blocker, implying a modulatory role for retinal dopamine in the response of the retina to light.

Project Description:

Additional Personnel Engaged on Project:

Daniel Weinberger	M.D.	Chief, SNN	NB, NIMH
Alec Roy	M.D.	Visiting Associate	CNB, NIMH

Protocol Number: 83-EI-230

Objectives: The primary interest is to characterize the role of the putative neurotransmitter dopamine on evoked potentials of the human retina. Our ancillary interest is the development of the electroretinogram as an objective physiological parameter in the therapeutic management and diagnosis of schizophrenic patients.

Methods Employed: Patients diagnosed with chronic schizophrenia are evaluated both on and off neuroleptic medication. The computer-averaged electroretinogram to Ganzfeld light stimuli is the primary tool to quantify retinal function.

In addition to this patient population, age and sex-matched controls are evaluated under identical conditions to obtain norms for comparison. These controls are also seen on a second visit so that test-retest reliability of the Ganzfeld electroretinogram can be evaluated.

Major Findings:

1. When the schizophrenic patients were off-medication, the implicit time of the cone a-wave was increased. This was observed for both dark- and light-adapted conditions suggesting a gross influence of neuroleptic-withdrawal on cone function. Furthermore, the first oscillatory potential, believed to originate in the inner nuclear layer, was also found to be delayed in dark- and light-adapted conditions. Since retinal dopamine has been found to have its highest concentration in this layer, a cause-effect relationship between dopamine blockade and oscillatory potential genesis in humans is implied. These results have led us to a pilot ERG study of patients with Parkinson's disease who are undergoing treatment with L-dopa at the Experimental Therapeutics Branch, NINCDS. These studies are now in progress.

Significance to Biomedical Research and the Program of the Institute: Previous papers have described the influence of neuroleptics on the electroretinogram of reptiles and lower vertebrates. There has been, however, a scarcity of clinical papers despite the high prevalence of schizophrenia in the general population. The data collected so far show a modulatory role of dopamine on the electroretinogram, suggesting that it can be used as an indirect measure of retinal dopamine activity.

Proposed Course: This project will continue.

NEI Research Program: Strabismus, Amblyopia and Visual Processing--Visual Processing and Amblyopia

Publications:

Karson CN, Jaffe MJ, Roy A, and de Monasterio FM: Electroretinography in schizophrenia. Biol Psych Abstr 39:58, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00123-04 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psycophysical Studies in Hemianopia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rafael C. Caruso	M.D.	Expert	CB, NEI
Others:	Myles J. Jaffe	O.D.	Staff Fellow	CB, NEI
	Kent E. Higgins	Ph.D.	Expert	CB, NEI
	Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI

COOPERATING UNITS *(if any)*

Department of Radiology, CC

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.08	0.95	0.13

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The visual function of patients with chiasmatic and retrochiasmatic lesions of the visual pathways is assessed with psychophysical tests. These include kinetic and static perimetry, color vision tests and spatial contrast sensitivity studies. The purpose of this study is to identify and develop tests to characterize the nature and evolution of visual loss in lesions that cause hemianopia.

Project Description:

Additional Personnel Engaged on Project:

Nicholas J. Patronas	M.D.	Staff Radiologist	CC, DR
Tomoyo Mikami	M.D.	Guest Worker	CB, NEI
Jacqueline Robinson		Secretary, SVP	CB, NEI

Protocol Number: 81-EI-108

Objectives: The aim of this project is to identify the patterns of visual loss and patterns of visual recovery in patients with chiasmal and retrochiasmal lesions of the visual system which determine hemianopic field defects and to develop the psychophysical tests best suited to monitor these functional changes.

Methods Employed: (1) Perimetry: The visual fields are explored with kinetic quantitative perimetry and static quantitative perimetry. In the latter procedure, the differential threshold for light perception is determined in a set of points in the visual fields to either side of the central vertical meridian, using a staircase method to identify the threshold. (2) Color vision: Central vision is estimated using the following methods: AO-HRR pseudoisochromatic plates, Farnsworth-Munsell D-15 panel, Farnsworth-Munsell 100-Hue test and the Nagel anomaloscope. (3) Spatial contrast sensitivity: The spatial contrast sensitivity function is determined using sinusoidal luminance gratings with spatial frequencies between 0.9 and 24 cycles/degree generated on an oscilloscope screen. A two-alternative temporal forced-choice technique is used for a criterion-free judgment of threshold visibility.

The psychophysical findings are correlated with the neuro-radiological estimation of the topography and size of the lesion.

Major Findings:

The effect of hemianopia on spatial contrast sensitivity was explored by studying the effect of hemifield stimulation in normal subjects. To ensure steadiness of fixation, the sinusoidal grating was stabilized by presenting it through the stimulus deflector unit of a Dual Purkinje Image Eyetracker. No contrast sensitivity loss, or only a minimal one, could be detected in these conditions. This result is analogous to the one seen in patients with hemianopia in whom the visual function of the remaining hemifield is intact. When the normal subjects viewed the stabilized grating through a neutral density filter, a marked middle and high spatial frequency loss was seen when one hemifield was stimulated. This loss is similar to that seen in patients with hemianopia in whom an involvement of the visual function of the remaining hemifield can be detected using static perimetry.

Significance to Biomedical Research and the Program of the Institute: Previous papers have described that patients with hemianopia show a contrast sensitivity loss proportional to the visual field loss in the affected hemifield. Our results suggest that central spatial contrast sensitivity remains within normal limits if at least one central hemifield maintains its normal function. This finding indicates that for the early diagnosis of central visual loss in patients with chiasmal or retrochiasmal lesions, contrast sensitivity measurements should be complemented with static perimetry of the central visual field.

Proposed Course: Psychophysical studies of visual function in chiasmal and retro-chiasmal lesions will be continued, introducing modifications of the techniques described that are expected to improve their diagnostic value.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications:

Caruso RC, Jaffe MJ, and Higgins KE: Contrast sensitivity in clinical and experimental hemianopia. Invest Ophthalmol Vis Sci 25(Suppl):177, 1984.

Koppelman MC, Jaffe MJ, Rieth KG, Caruso RC, and Loriaux DL: Hyperprolactinemia, amenorrhea and galactorrhea. A retrospective assessment of twenty-five cases. Ann Intern Med 100:115, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00144-03 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Clinical Electrophysiology of the Visual System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rafael C. Caruso	M.D.	Expert	CB, NEI
Others:	F. M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
	Kent E. Higgins	Ph.D.	Expert	CB, NEI
	Tomoyo Mikami	M.D.	Guest Worker	CB, NEI
	Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI
	Jacqueline Robinson		Secretary, SVP	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.98	0.85	0.13

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Visual evoked responses are recorded in normal volunteers and in patients with lesions of the retina, optic nerves, optic chiasm, optic radiations and visual cortex. Both pattern stimuli and unstructured stimuli are used. The recordings are used for diagnostic purposes and to provide an objective assessment of visual function in these conditions. These data are correlated with the results of psychophysical tests of visual function.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 82-EI-55

Objectives: The aims of this project are to characterize the normal electrical activity of the human visual system and to analyze the patterns of its alteration in lesions of the visual pathways.

Methods Employed: Visual evoked responses are elicited by pattern reversal stimuli (checkerboards displayed on a television screen or sine-wave gratings generated on an oscilloscope screen) and by unstructured stimuli (achromatic and chromatic flashes). Recordings of the electrical activity of the retina and the occipital lobes obtained after each stimulus presentation are digitized and averaged to isolate the components of the visual evoked response.

Major Findings:

1. A complete functional assessment of the visual system with electrophysiological techniques involves the description of the electrical activity of the retina, the retinogeniculate pathway, the lateral geniculate body, the geniculocortical pathway, and the visual cortex. This objective has been partially achieved by three procedures that have proved their clinical value: the electrooculogram, an estimation of the standing potential generated at the level of the retinal pigment epithelium; the electroretinogram, which reflects the electrical activity of the outer layers of the retina; and the cortical visual evoked potential, which assesses the function of the visual cortex and the optic radiations. A significant segement of the visual pathways remains, however, unexplored by these methods.

The pattern electroretinogram (PERG), or pattern evoked retinal response, which probably reflects the electrical activity of the ganglion cell layer of the retina and distal segment of the optic nerve, has recently emerged as a potentially useful diagnostic technique for the study of the disorders of the optic nerve. We have developed a system for the recording of the PERG and plan to use this method in the management of optic neuropathies. The clinical value of the subcortical visual evoked response, an electrical potential thought to reflect the functional activity of the lateral geniculate nucleus and/or its afferent and efferent pathways, has not been adequately determined. We plan to adapt our recording techniques to detect the subcortical VER and study its pathological alterations in diseases of the visual system.

2. The effect of cyclosporine therapy on the visual evoked responses of patients with multiple sclerosis and uveitis was studied. Before cyclosporine therapy, transient visual evoked potentials showed a markedly increased latency of the P100 peak, and a moderate reduction of its amplitude. Six months after the treatment was initiated, analogous recordings showed a marked reduction of the latency of the P100 peak and a moderate increase of its amplitude. The electrophysiological findings were associated with an improvement of the uveitis and the systemic neurologic status.

Significance to Biomedial Research and the Program of the Institute: Electrophysiological techniques provide non-invasive diagnostic methods for the management of ophthalmological and neuro-ophthalmological disorders. The ongoing research is expected to provide information about normal responses generated by areas of the visual system not explored by the classical electrodiagnostic methods and about the alterations of these responses in pathological conditions.

In patients with uveitis, it is frequently important to decide whether an involvement of the optic nerve is a factor in the etiology of the visual loss. In this particular case, the visually evoked response proved to be a sensitive indicator of the presence of an optic neuropathy. The reduction of the latency-to-peak of the response is remarkable since latency changes in multiple sclerosis are usually persistent and stable. The possible value of cyclosporine in the treatment of optic neuropathies associated with uveitis warrants further study.

Proposed Course: Recordings of potentials evoked by visual stimuli in patients and in normal subjects will be continued, introducing modifications of the techniques described that are expected to improve their diagnostic value.

NEI Research Program: Strabismus, Amblyopia and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications:

Caruso RC, Higgins KE, and Schefrin BE: Visual evoked responses in clinical and experimental oscillopsia. Invest Ophthalmol Vis Sci 24(Suppl):60, 1983.

Nussenblatt R, Palestine A, Chan C, Breen L, and Caruso R: Improvement of uveitis and optic nerve disease by cyclosporine in a patient with multiple sclerosis. Am J Ophthalmol 97:790, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00006-13 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Measurement of Color Vision

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB, NEI
Others:	Monique Roy	M.D.	Visiting Scientist	CB, NEI
	Fred C. Chu	M.D.	Senior Staff Fellow	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.80	0.80	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In addition to the color tests, measurements of rod and cone thresholds are made on some patients, particularly those beginning or receiving medication for lupus erythematosus or rheumatoid arthritis. Patients from studies on diabetic and sickle cell retinopathy and their age-matched normal controls are given four kinds of color tests. Some of the tests are repeated at later dates for confirmation of findings or possible correlation with changes in the disease state.

Tests were conducted on 243 patients, including some normal controls during the year.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 80-EI-08

Objectives: There are two interlocking parts to this project, one being the confirming or refuting of any reported correlation of specific types of color defects with certain disease entities, or discovering new ones that may exist. Secondly, we expect in some cases to be able to monitor the progress or regress of disease conditions by serial measurements of the extent of a color vision defect.

Methods Employed: Under standard light conditions, subjects are tested with the Farnsworth-Munsel D-15 and 100-Hue tests, the Lanthony Desaturated D-15 test, and the Chromograph.

Major Findings:

Relatively few acquired color defects are disclosed by the Panel D-15 test. When they are severe enough to be shown by two or more errors in the Panel D-15, a color defect of some sort is invariably shown by one or more of the other tests. If only one error is made, it is difficult to decide whether the error is due to poor discrimination, carelessness, haste, boredom, or frustration, even if the same type happens to be indicated by another kind of test.

The Lanthony Desaturated test suggests the existence of many color defects, even among subjects considered to be normal by the Panel D-15 and the 100 Hue tests. Some of these errors are probably due to the personality or mood factors mentioned above, but some of them are consistently confirmed by the chromograph, especially if one considers the relatedness of blue and yellow defects.

The 100-Hue test is subject to many interpretations, partly because of the time and patience required for its performance. Some subjects are overwhelmed by the similarity of the 21 buttons and become almost frantic in their efforts to arrange them in order. Others are very systematic and meticulous, sometimes wanting an hour to arrange the whole set. Some subjects express annoyance, frustration, or boredom. Another possible source of error is due to the fact that buttons near the ends of different trays are scored with respect to each other, even though there is no opportunity for them to be observed together. It would not be practical to present all 85 buttons in one random assortment, so there is no obvious solution to this problem. Repeating the test should provide some reassurance or confirmation of findings, but performance and scoring are so tedious that both subject and examiner are usually reluctant to undertake repetitions. Furthermore, a second or third test rarely shows the major errors to be in the same axis, if indeed an axis can be discerned. If an axis is observed which does not correspond with the accepted position for protanomaly (red), deuteranomaly (green) or tritanomaly

(blue), the classification is unclear because the larger errors (long points) indicate areas of confusion rather than thresholds of discrimination to specific colors. Total error score may indicate sensitivity to color in general, if no major axis is observed, or it may be more related to the care with which the test was performed. At any rate, it is not a reliable indicator for specific types of color defects of the acquired type.

The chromograph requires less time and concentration while providing more specific information than either of the other tests. Thresholds found with the small test area (about one degree at 30 inches) are usually more effective in plotting color defects than are those found with the large test area. If a defect is suspected, it can be checked repeatedly for confirmation in a matter of seconds, which is not possible with the other tests. The chromograph, with its capacity for producing all colors, reveals the inadequacy of the only type names commonly used, namely protanomaly, deuteranomaly, and tritanomaly.

While the Lanthony test appears to be more sensitive to subtle defects than either the Panel D-15 or the 100-Hue, it may also be more specific as to color than the 100-Hue test. In most cases where a slight defect is noted with the Lanthony test, it is more definitely shown with the chromograph. Allowance must be made for occasional chance or accidental errors with Lanthony and its confusion or mixing of yellow and blue. There is a significant number of cases where the Panel D-15, Lanthony, and 100-Hue tests all appear normal and the chromagraph consistently shows an appreciable defect.

Significance to Biomedical Research and the Program of the Institute: In clinical work it is important to know which tests will be useful or appropriate for different types of patients since physical, mental, and visual disabilitites will make any test more difficult and less reliable.

Proposed Course: The project should be continued for the purposes of correlating subtle color defects with specific disease and drug entities, and monitoring a disease state when appropriate.

NEI Research Program: Retinal and Choroidal Diseases-- Retinal Organization, Neurotransmission, and Adaptation

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00121-04 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Spatial Contrast Sensitivity Studies in Retinal and Neuro-ophthalmologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kent E. Higgins	Ph.D.	Expert	CB, NEI
Others:	F. M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
	Rafael C. Caruso	M.D.	Expert	CB, NEI
	Monique S. Roy	M.D.	Visiting Associate	CB, NEI
	Thomas A. Margevicius	O.D.	Guest Worker	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.85	0.42	0.43

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Spatial contrast sensitivity was used to assess losses or changes in overall visual resolution in patients having a variety of toxic, inflammatory, degenerative, or congenital retinal and neuro-ophthalmological disorders of the visual system. A criterion-free forced-choice psychophysical procedure was used throughout, since this method was found to produce minimal false positive or false negative diagnoses at initial test and to minimize spurious changes in sensitivity with repeated testing. For detecting early losses and monitoring changes in central-field visual resolution, this test continued to be superior to conventional visual acuity testing.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI
Roger W. Hynes	A.A.S.	Health Technician	CB, NEI
Jacqueline D. Robinson		Secretary, SVP	CB, NEI

Protocol Numbers: 81-EI-34, 84-EI-109

Objectives: The objectives of this project are to determine whether specific visual disorders produce specific contrast sensitivity deficits and to determine the utility of contrast sensitivity measurement for monitoring changes in overall visual resolution during treatment for various ocular disorders.

Methods Employed: Overall spatial contrast sensitivity was measured in the central field of patients undergoing treatment for ocular disorders of diverse etiology (e.g., diabetes, gyrate atrophy, and uveitis). To control for possible fluctuations in the patient's criterion for judging grating visibility, a computer-controlled forced-choice psychophysical procedure was used for all measurements. Patient contrast sensitivity deficits were defined by reference to age-normative data obtained using the same apparatus and procedure for testing normal volunteers. In addition, limited studies were begun using an improved and abbreviated version of the forced-choice method which reduced patient testing time by 1/3.

Major Findings:

1. In some diabetic patients having normal visual acuity, spatial contrast sensitivity test results showed significant week-to-week fluctuations in central vision that were not correlated with blood sugar level. Sample size was not large enough to provide an estimate of the generality of these findings.

2. Following administration of cyclosporine for the medical treatment of uveitis, spatial contrast sensitivity was found to be a more sensitive and reliable measure of changes in visual resolution than conventional visual acuity testing.

3. Spatial contrast sensitivity testing showed differences in visual function among patients having similar visual field losses as defined by Goldmann perimetry.

Significance to Biomedical Research and the Program of the Institute: Visual acuity tests have provided a standard means of assessing central field resolution for fine spatial detail. In recent years, however, the limitations of visual acuity testing have become increasingly apparent. Such limitations have led to an increasing reliance on spatial contrast sensitivity measurement as a means of obtaining an overall estimate of visual resolution for coarse as

well as fine spatial detail. Nonetheless many of the same biases which could produce spurious visual acuity test results have also been incorporated into some methods for clinical spatial contrast sensitivity measurement (e.g., "practice effects"). Results obtained using the criterion-free forced-choice procedure have shown the value of measuring spatial contrast sensitivity under conditions which control for such biases such that the measured sensitivity values reflect visual function (or changes therein).

Proposed Course: Studies of spatial contrast sensitivity will be continued with a greater emphasis being given to the use of the newly developed system. This new system provides greater flexibility in the selection of test field size and mean luminance and, importantly, promises to cut testing time by 1/3 without sacrifice of precision or reliabilty.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing

Publications:

Higgins KE, Jaffe MJ, Coletta NJ, Caruso RC, and de Monasterio FM: Spatial contrast sensitivity: Importance of controlling the patient's visibility criterion. Arch Ophthalmol 102:1035, 1984.

Caruso RC, Jaffe MJ, and Higgins KE: Contrast sensitivity in clinical and experimental hemianopia. Invest Ophthalmol Vis Sci 25(Suppl):177, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00175-02 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Normative Studies of Spatial Contrast Sensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kent E. Higgins	Ph.D.	Expert	CB, NEI
Others:	F. M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
	Myles J. Jaffe	O.D.	Staff Fellow	CB, NEI
	Rafael C. Caruso	M.D.	Expert	CB, NEI
	Thomas A. Margevicius	O.D.	Guest Worker	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.82	0.35	0.47

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Age-referenced test and retest norms for spatial contrast sensitivity were determined for adult subjects ranging in age from 21-70 years of age using two psychophysical methods, a method of adjustment and a criterion-free two-alternative, temporal forced-choice procedure. Both methods showed a decline in contrast sensitivity with age, with the forced-choice method showing the smallest loss with age. The larger loss obtained using the method of adjustment can be explained by probable criterion differences between younger and older observers. Testing was initiated for subjects in the 5 to 20 year age range. However, sample size was not large enough to permit statistical analysis of the results for this age range. In addition an abbreviated version of the original forced-choice procedure was developed. Standardization of this new system was begun and preliminary results indicate that it will reduce total patient test time by approximately one-third.

Project Description:

Additional Personnel Engaged on Project:

Patricia A. Mercer	B.S.	Clinical Technician	CB, NEI
Roger W. Hynes	A.A.S.	Health Technician	CB, NEI
Jacqueline D. Robinson		Secretary, SVP	CB, NEI

Protocol Number: 81-EI-34

Objectives: The objectives of this project are to compare the reliability and efficiency of different psychophysical methods for the clinical measurement of spatial contrast sensitivity, to determine the magnitude of age-related changes in normal spatial contrast sensitivity, and to provide age-referenced normative test data for the evaluation of spatial contrast sensitivity in NEI eye clinic patients.

Methods Employed: Two psychophysical methods were used, a method of adjustment and a forced-choice procedure. The former method was used because it is representative of methods currently used for clinical contrast sensitivity measurements insofar as it does not permit specification of the threshold criterion used by the patient in judging grating visibility. The latter method consists of a criterion-free, two-alternative, temporal forced-choice procedure which determines the contrast required for detection of a grating patttern on a fixed percentage (criterion) number of presentations. Spatial contrast sensitivity was measured at 9 spatial frequencies, ranging from 0.75-21.0 cycle/degree, and in subjects ranging in age from 5 to 70 years. In addition, preliminary data were collected using a newly-completed forced-choice test system which measures contrast sensitivity at 6 instead of 9 spatial frequencies.
All normal subjects were screened to ensure that they had normal visual characteristics. Cycloplegia was used to minimize accommodative fluctuations, and an artificial pupil was used to standardize pupil size. Contrast sensitivity was tested twice in each patient using both methods to determine the stability of test and retest data.

Major Findings:

1. Normal aging produced a middle and high spatial frequency loss in spatial contrast sensitivity, with the method of adjustment showing approximately twice the loss observed using the criterion-free forced-choice method. With the younger age groups (21-30 yrs), both psychophysical methods yielded similar mean sensitivities. For the older age groups, the mean sensitivity was typically higher using the forced-choice method. This suggests that the larger loss in contrast sensitivity observed with the method of adjustment may reflect nonvisual as well as visual changes with age.

2. Although testing was extended to include normal subjects as young as 5 years of age, the sample size was not large enough to permit evaluation of possible contrast sensitivity changes from the preteen to teenage years.

3. For each age group, sample standard deviations were less using the forced-choice method than using the method of adjustment.

4. Approximately one-half of the normal subjects showed large and spurious shifts in sensitivity from test to retest using the method of adjustment. Shifts of this magnitude were not observed using the forced-choice method.

5. Preliminary results indicate that the newly constructed forced-choice test system is as reliable as the original system while reducing total test time by one-third.

6. No sex differences in contrast sensitivity were observed.

Significance to Biomedical Research and the Program of the Institute: Previous studies provide conflicting evidence concerning the effect of normal aging on spatial contrast sensitivity, with some reporting an overall loss at all spatial frequencies and others reporting only a mid and high spatial frequency loss. In the present study, it is important to note that the type and magnitude of loss observed depended on the psychophysical method used to measure contrast sensitivity. When the criterion being used by the patient for judging grating visibility was not controlled (method of adjustment), an overall loss in sensitivity was observed. When, however, the criterion-free forced-choice method was used, a smaller and predominantly high spatial frequency loss was observed. These results therefore indicate the importance of using criterion-free methodology to provide not only accurate estimates of the effect of aging on vision but also accurate estimates of visual loss due to a superimposed ocular disorder.

Proposed Course: These studies will be continued, with particular attention being given to the collection of normative data for the various age groups using the new test system developed during the past year. The greater speed and flexibility of the new system will make it possible to test patients more efficiently and to test patients with more severe visual loss.

NEI Research Program: Amblyopia, Strabismus and Visual Processing

Publications:

Higgins KE, Jaffe MJ, Caruso RC, and de Monasterio FM: Aging and spatial contrast sensitivity. J Opt soc Am 73:1939, 1983.

Higgins KE, Jaffe MJ, Coletta NJ, Caruso RC, and de Monasterio FM: Spatial contrast sensitivity: Importance of controlling the patient's visibility criterion. Arch Ophthalmol 102:1035, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00174-02 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Simulation of Visual Impairments in Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kent E. Higgins	Ph.D.	Expert	CB, NEI
Others:	Rafael C. Caruso	M.D.	Expert	CB, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.30	0.30	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To determine the relationship between spatial contrast sensitivity and visual field loss in an otherwise normal eye, contrast sensitivity was measured under conditions simulating varying types and degrees of central visual field loss. Very small artificial scotomata produced middle and high spatial frequency losses in sensitivity. For larger scotoma, the type of loss obtained depended on temporal factors associated with grating presentation. A predominantly high spatial frequency loss was observed if grating contrast was turned on and off gradually. An additional low frequency loss was obtained under conditions in which grating contrast was turned on and off abruptly to produced sharp temporal transients. Under these transient conditions, larger central scotomata produced larger losses in contrast sensitivity with the rate of loss being larger for the higher spatial frequencies.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 81-EI-34

Objectives: The effect of pathologic visual field loss on spatial contrast sensitivity is only poorly understood. One obvious effect of a real scotoma is to decrease the effective area of the stimulus field being viewed by a patient. To determine whether this factor is sufficient to explain the observed contrast sensitivity deficit in a patient, it is necessary to determine the relationship between spatial contrast sensitivity and visual field loss in the normal eye under artificial scotoma conditions.

Methods Employed: Spatial contrast sensitivity was measured in normal subjects with and without artificial central scotomata of varying diameters. The scotomata were retinally stabilized by using an SRI Double Purkinje-image Eyetracker. A two-alternative, temporal forced-choice psychophysical procedure was used to minimize subject criterion fluctuations. These measurements were repeated under three different sets of conditions. In the first, the contribution of temporal transients associated with grating presentation was minimized by turning grating contrast on and off gradually. In the second condition, transients were introduced by presenting the grating patterns with abrupt temporal onsets and offsets. In the third condition, temporal transients were amplified by temporal contrast-reversal of the grating patterns. For this third condition, the initial measurements were made using the psychophysical method of adjustment and these results were later replicated using a forced-choice psychophysical procedure.

Major Findings:

1. Artificial central scotomata of less than about 2 degrees diameter produced a predominantly high frequency loss in spatial contrast sensitivity, irrespective of the temporal method used for grating presentation.

2. For larger artificial scotomata, the type of loss obtained depended on the type of temporal presentation used. With gradual onset and offset of grating contrast, a predominantly high spatial frequency loss was observed. When, however, temporal transients were introduced by either turning grating contrast on and off abruptly or amplified by the contrast-reversal technique, an additional loss in low spatial frequency sensitivity was observed.

3. Under conditions which do not minimize temporal transients, the loss in sensitivity increased with scotoma size with the rate of loss being greater at the higher spatial frequencies. Preliminary results obtained under conditions which minimized temporal transients yielded similar trends except that a larger central scotoma was necessary to produce a measureable low spatial frequency change in sensitivity.

Significance to Biomedical Research and the Program of the Institute: Traditionally the central field is thought to be concerned with mediating fine-detail vision. Loss of the central field is accordingly expected to produce decreased visual acuity and a loss in high spatial frequency contrast sensitivity. These results indicate that whether or not one obtained a predominately high frequency loss or an overall loss at all spatial frequencies may depend importantly on the contribution of temporal factors. In clinical terms this means that temporal factors are likely to play an important role in determining whether a patient with a central scotoma will manifest a loss at low as well as high spatial frequencies. Because of the potential importance of lower spatial frequency sensitivity for such everyday activities as orientation and mobility, accurate assessment of low frequency spatial contrast sensitivity is likely to become increasingly important for management of those patients having severe visual losses.

Proposed Course: To date, most of the experiments have dealt with variations in scotoma size for a fixed grating field size and with temporal factors associated with grating presentation. Future experiments will examine the effect varying grating-scotoma sizes to determine the effect of compensating for the cortical magnification factor and with variations in mean luminance level.

NEI Research Program: Visual Impairment and Its Rehabilitation

Publications:

Higgins KE, Caruso RC, Coletta NJ, and de Monasterio FM: Effect of artificial central scotoma on the spatial contrast sensitivity of normal subjects. Invest Ophthalmol Vis Sci 24:1131, 1983.

Caruso RC, Jaffe MJ, and Higgins KE: Contrast sensitivity in clinical and experimental hemianopia. Invest Ophthalmol Vis Sci 25(Suppl):177, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00063-06 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acquired and Congenital Color Vision Deficiencies: Mechanisms and Diagnosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kenneth B. Knoblauch	Ph.D.	Staff Fellow	CB, NEI
Others:	Francisco M. de Monasterio	M.D., D.SC.	Head, SVP	CB, NEI
	Kent E. Higgins	Ph.D.	Expert	CB, NEI
	Rafael C. Caruso	M.D.	Expert	CB, NEI

COOPERATING UNITS *(if any)*

College of Optometry, University of Houston; Optical Radiation Branch, Center for Devices and Radiological Health, FDA (M. Waxler)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project involves the study of cone function in cases of color vision defects, with special emphasis on acquired deficiencies. Human subjects will be examined with both electrophysiological and psychophysical tests. Experimental studies are carried out in non-human primates.

Project Description:

Additional Personnel Engaged on Project:

Roger W. Hynes	A.A.S.	Health Technician	CB, NEI

Protocol Number: 79-EI-92

Objectives: To characterize and document color vision abnormalities mediated by dysfunction of blue-, green- and/or red-sensitive cones or of their corresponding pathways, and to examine mechanisms of acquired deficiencies.

Methods Employed: Color vision is examined on the basis of a battery of psychophysical tests (increment thresholds, field and test spectral sensitivities, spectral luminosity, saturation discrimination tests), and electrophysiological studies of cone-mediated potentials, intense blue light exposure in non-human primates and intravitreal dye injections in macaque eyes.

Major Findings:

1. Light-induced damage of blue-sensitive cones of macaque retina. The results of a series of anatomical studies of macaque retinas suggests that exposure of the retina to intense short-wavelength lights results in damage selectively to the blue-sensitive cones. The retinal distribution of damaged receptors is consistent with that of the blue-sensitive cones. Psychophysical studies are being performed on animals exposed to blue-light to confirm the suspected dysfunction of blue-cone mediated responses. These studies may provide a useful animal model of acquired tritanopic defects of color vision.

2. Human blue-sensitive cone function and retinal aging. We have developed a simple electroretinographic system to measure potentials mediated by blue-sensitive cones. We have already measured peak amplitude and implicit time of ERG b-waves mediated respectively by signals from dark-adapted rods, dark-adapted blue cones, and dark-adapted red and green cones in male and female normal volunteers of various ages. We plan to continue the measurements of the ERG b-waves associated with the various receptor systems in the human eye, but we are also adding measurements that allow for estimates of the lens and optic media transmission factors to be determined individually for each eye tested. These estimates will permit a more accurate determination of the contribution of pre-retinal transmission factors to age-related changes in the various potentials than can be obtained by using average lens transmission data.

3. Increment threshold measurements of visual mechanisms and wavelength saturation discrimination. We are building a system that will have the flexibility to allow for measurment of increment thresholds, field and test spectral sensitivities, and flicker luminosity. These psychophysical measures

provide data on the integrity of adaptive processes and color vision mechanisms.

Along these same lines, we have built a device to provide a quick measure of the integrity of blue-sensitive cone functioning. This device exploits a phenomenon in which there is a short-term loss in sensitivity in the blue-sensitive cone system at the onset of light-adaptation with moderately intense yellowish lights. We expect that patients with blue-sensitive cone dysfunction will show a prolonged recovery of sensitivity as compared to those with normal function. This test will provide a quick screening method for yellow-blue defects, and may provide a sensitive quantitative index of changes in the blue-sensitive cone system over time.

We also plan to measure wavelength saturation discrimination functions for normal and color defective observers. These measurements will determine the loci of neutral points (i.e., wavelength regions that are confused with achromatic lights) in acquired color vision deficiencies. This information will permit the determination of color confusion loci in such patients. These values are expected to help both with the classification and diagnosis of acquired deficiencies and should provide information on ways to modify conventional color vision tests and to develop new ones.

4. Assessment of color vision tests. We are beginning to examine the performance of normal and color-defective individuals using modifications of standardized color vision tests. The Farnsworth Panel D-15 and 100-Hue test are being examined at a variety of illumination levels, and under variations of the color temperature of the illumination light. Such modifications are known to eventually result in an abnormal test result in normal subjects. The aim of these studies is to determine those conditions which provide the largest differences in the scoring of subjects with normal and abnormal color vision. We are also examining a version of the D-15 test that utilizes large color testing chips. These may be of greater use in testing patients with macular defects or low-vision problems.

Significance to Biomedical Research and the Program of the Institute: These studies contribute to our understanding of the mechanisms of acquired color vision defects in cases of retinal insult or disease. In addition, some studies may provide information on the development of new and simple tests for the diagnosis and classification of acquired color vision defects.

Proposed Course: These studies will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction.

Publications:

Higgins KE, Brooks DN, Gottschalk G: Tritan pedigree without optic nerve atrophy. Am J Optom Physiol Opt 60:964, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00186-01 CB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ophthalmic Manifestations of Kallman's Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Myles J. Jaffe	O.D.	Staff Fellow	CB, NEI
Others:	Georgia Chrousos	M.D.	Senior Staff Fellow	CB, NEI
	Victor Matsuo	M.D.	Staff Fellow	CB, NEI

COOPERATING UNITS *(if any)*

Section on Reproductive Biology, NICHHD (R. Sherins)

LAB/BRANCH

Clinical Branch

SECTION

Section on Visual Processing

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.55	0.55	0.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with Kallman's syndrome are known to have multiple midline defects. In this study, two additional midline defects, both ophthalmic, have been identified to occur with sufficient frequency that these signs should be evaluated routinely. The first is a transillumination defect of the iris inferior quadrant which is more subtle and far more common than a complete iris coloboma. The second is dysmetric horizontal saccades which are attributed currently to a midline defect of the cerebellum. These findings provide a more complete characterization of the identifiable midline defects of patients with Kallman's syndrome.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Patients who have hypothalmic hypogonadism and midline defects, Kallman's syndrome, were suggested in 1944 to have anosmia and color vision deficiency as part of the disease triad. DEB, NICHHD patients with this disease were sent as consults to the Clinical Branch to have their pattern of presumed color vision loss characterized so that appropriate tests of color vision could be included in their diagnostic work-up.

Methods Employed: Patients with diagnosed Kallman's disease underwent a routine ophthalmic exam in addition to extended color vision testing, visual fields, optokinetic testing, video recordings of eye movements and anterior segment photography.

Major Findings:

We found that patients with Kallman's disease have an incidence of inherited color vision loss that is consistent with that of the general population. Thus, color vision deficiency may not be included in the diagnostic triad. However, other ophthalmic manifestations of this disease have been identified. These include iris transillumination defects of the inferior quadrant and dysmetric saccades. The former is consistent with an ocular midline defect, while the latter may represent a midline defect in the cerebellum.

In patients with Kallman's syndrome, multiple midline structures are known to be involved, including the hypothalamus, olfactory bulb, palate, and penis. Although coloboma of the iris, retina, and optic nerve have been individually reported, they are not seen consistently and were not observed in any of the 43 patients evaluated this year. By comparison, the inferior quadrant iris defect was often identified indicating that the globe should be routinely studied as part of the Kallman's workup.

Similarly, a pattern of eye movement disorders has been recognized in this syndrome that implicates the midline of the cerebellum to be not uncommonly involved. This again suggests routine evaluation of eye movements be included in the Kallman's workup.

Significance to Biomedical Research and the Program of the Institute: Two additional midline structures have been identified to be involved in Kallman's, and significant color vision loss has been excluded. This information provides a more complete characterization of Kallman's syndrome and may be of value in future investigations into the embryogenesis of the multiple midline defects identified in this syndrome.

Proposed Course: This project will not be continued.

NEI Research Program: Strabismus, Amblyopia and Visual Processing

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00001-02 CB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Activity labeling with 2-deoxyglucose of the visual system of primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Stanley J. Schein	M.D., Ph.D.	Expert	CB, NEI
Others:	Francisco M. de Monasterio	M.D., D.Sc.	Head, SVP	CB, NEI
	Edna P. McCrane	B.S.	Biologist	CB, NEI
	S. Spencer		Student Scientist	CB, NEI
	David Kostick		Normal Volunteer	CB, NEI

COOPERATING UNITS *(if any)*
Laboratory of Neuropsychology, NIMH (R. Desimone)

LAB/BRANCH
Clinical Branch

SECTION
Section on Visual Processing

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.58	0.80	0.78

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aims of this project are to establish the scientific basis for obtaining fine-resolution 2-deoxyglucose activity labeling in primate brain and retina, to develop ancillary methods to assist interpretation of the resultant autoradiograms, and to apply these advances to biological studies. Investigations of the chemistry of withdrawal of label from tissue have revealed that the mechanism is the process of Desorption. This theoretical description indicates that label stays in place or leaves the tissue but does not wander. As a practical matter, conditions and solvents can now be specified for minimal loss of label during the various steps. Indeed, we find that 98% of the label is retained in place by this new fine-resolution method. Double label trials, taking rate of uptake from plasma into account, reveal the presence of a lag period between change in neural activity and change in metabolic activity. Finally, fine-resolution deoxyglucose autoradiograms of the marmoset monkey retina show numerous layers, including a very active retinal pigment epithelium.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To establish the scientific basis for technology needed to study the neural organization underlying visual processing in the visual system of primates, specifically the retina and the visual cortex.

Methods Employed: Activity labeling with 2-deoxyglucose, computer graphics stimulation, image processing technology, liquid scintillation counting, and autoradiography.

Major Findings:

We have been developing and studying the scientific basis of a new technique aimed at improving the resolution of the quantitative deoxyglucose method for autoradiographic mapping of glucose utilization. Each of the steps proposed in the new method must operate within severe constraints, viz. the large size of the tissue involved, the necessity of quantitative retention and of strict localization of the water-soluble label. We have attacked the last remaining problems this year: Fixation of tissue which prevents leakage of label, the physical chemistry of withdrawal of label by solvents, double-labeling, and the handling of retina.

1. Perfusion fixation is the first step in the processing of tissue. To evaluate the effect of perfusion and of different compositions of fixative, an assay has been developed to compare tracer concentrations in fellow hemicortices after differential treatment. The compositon of the fixative we have been using is based on its ability to fix neural retina without causing cells to become leaky. The new assay indicates that this fixative is quantitatively benign, producing no loss of label from the brain.

2. Over the last years, withdrawal of tissue label by solvents has been assayed by measuring the radioactivity washed out from a piece of 2DG-labeled brain. We established that solubility is not a useful concept in explaining the results; instead, there appears to be an "accessible pool" of label, all of which can be withdrawn by a particular solvent. In addition, we found that the size of the accessible pool depends critically on solvent chemistry: Increasing ability to hydrogen bond increases withdrawal; increasing dielectric constant increases withdrawal. These results dictate the use of different classes of solvent.

3. Dissolved water increases withdrawal of label in direct proportion to aqueous concentration. For just 8% water, 50% of the label is withdrawn. Water miscible solvents are required for the freeze-substitution step, which accomplishes removal of water from tissue at low temperature.

4. Withdrawal of water depends on temperature, declining as the temperature is reduced, in a lawful fashion suggesting an "activated" process.

5. The kinetics of withdrawal are logarithmic with time for all conditions of temperature and choice of solvent. Logarithmic kinetics can be obtained by assuming a flat distribution of activation energies for the process of withdrawal. We are the first to solve the integral that models the process.

6. Desorption is a physical chemical process that is consistent with all of the experimental findings. The practical value of these studies is that they permit rational choice of solvents and conditions under which tissue may be exposed to solvents, producing extremely low, but predictable losses of tissue label. The value of the theory is in permitting the conclusion that label either remains in place or is washed out, but it does not wander.

7. Interpretation of 2DG autoradiograms is not a trivial mater. So-called selective stimulation by a rich, colored stimulus with no black and white, for example, should activate nearly all visual mechanisms, rather than stimulate a single specific mechanism. Double-labeling with 2DG would permit comparison within the same cortical space of spatial codes for different stimulus configurations. We had devised techniques for separation of 3-H and 14-C maps into separate autoradiograms. A test of the method employed occlusion of one eye during the first labeling epoch, and occlusion of the other eye during the second labeling epoch. Successful double labeling would be indicated by high labeling of one set of ocular dominance columns by one label with high labeling of the other ocular dominance columns by the other label. The schedule of injections and stimulation must take into account the rate of uptake of label from the plasma: Change in metabolic activity lags behind change in neural activity.

8. The marmoset monkey retina was autoradiographed at 7 microns resolution. Numerous layers are visible, including a double layer in photoreceptors (ellipsoids of inner segments and nuclei). Single cells were visible, especially in the retinal ganglion cell layer. Finally, the most highly labeled layer is the retinal pigment epithelium.

Significance to Biomedical Research and the Program of the Institute: Understanding the organization of the visual system of non-human primates is valuable for understanding the human visual system. Functional descriptions of cortical organization in monkeys should, in themselves, provide insight on human visual deficiencies. More specifically, positron-emitter labeled 2-deoxyglucose is already in use for non-invasive activity labeling in human. The resolution of such labeling, however, is quite poor (in the 4-6 mm range),

and there are restrictions in the number of times the test can be used in the same patient within a single year. Clearly, optimization of stimuli to maximally and differentially stimulate functional areas is of special importance.

Proposed course: This project will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia--Structure and Function--Cell and Systems.

Publications:

Schein SJ and de Monasterio FM: Fine-resolution, quantitative activity-labeling of macaque brain with 2-deoxyglucose. Neurosci Abstr 9:154, 1983.

LABORATORY OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE CHIEF, LABORATORY OF MOLECULAR AND DEVELOPMENTAL BIOLOGY

Joram Piatigorsky, Ph.D.

This third year of the Laboratory of Molecular and Developmental Biology has been very productive and one of entrenchment. All of our laboratory space has finally been obtained, and at the time of writing, the renovations are being completed. The laboratory was also reviewed by the Board of Scientific Counselors for the first time in June. The most important developments were, however, the work itself. Each of the four groups progressed appreciably in their research efforts, and a sense of excitement was in the air on numerous occasions. Highlights of our studies are given below.

My group studying the crystallin genes has advanced considerably. Various approaches for understanding the regulation of δ-crystallin gene activity have started to merge. Last year we described the δ-crystallin gene locus at the level of electron microscopy and restriction enzyme analysis; the results showed the existence of two linked genes with similar structure, each containing at least 17 introns. This year we characterized the 5' regions and the associated flanking sequences (promoter regions) of both genes by nucleotide sequencing. This provided the basis for future studies on their expression. Moreover, the results indicated that the more active of the two genes (δ1) had structural features (viral core-enhancer-like sequences and a CCAAT box) that were missing from the less active gene (δ2). These features might account for their differential activity. The δ1 promoter was found more active than the δ2 promoter in cell-free transcription studies, paving the way for in vitro analysis of these two crystallin gene promoters. An important advance was the development of a tissue culture system for the study of crystallin gene promoters. Primary explants of embryonic chicken lens epithelia were found to take up and express recombinant pSVO expression vectors we have made containing the bacterial chloramphenicol acetyl transferase (CAT) gene fused to crystallin promoters. Particular attention was given to the murine αA-crystallin gene promoter. Tissue-specific expression of this promoter was demonstrated, and at least 400 base pairs of 5' flanking sequence were found for maximum function of the gene under our experimental conditions. More important, these experiments have established that this approach will be useful for detailed analysis of the DNA sequences required for proper regulation of the crystallin genes. In addition to the functional studies, a number of other crystallin genes have been isolated which are being characterized at the sequence level. Thus, the crystallins are being defined at the level of their genes and the foundation is being created to understand their evolutionary relationships and regulated expression during development.

We have had very productive collaborative projects this year. The murine γ-crystallin gene family is being extensively characterized with Drs. Martin Breitman and Lap-Chee Tsui at the Hospital for Sick Children in Toronto, Canada. A study demonstrating selective loss of γ-crystallin synthesis and γ-crystallin mRNAs in the hereditary Fraser mouse cataract has been completed with Dr. Anthony Garber and Reney Gold at the University of Toronto, Canada. A new crystallin from the turtle lens (τ-crystallin) has been extensively

purified and characterized in collaboration with Dr. Joseph Horwitz at the Jules Stein Eye Institute in Los Angeles, California, and Dr. Leah Williams at the University of West Virginia in Morgantown, West Virginia. Unexpectedly, it appears as if τ-crystallin may be related to δ-crystallin and may allow us to trace the origins of δ-crystallin to organisms predating the reptiles. With Dr. George Inana and Ms. Deborah Carper of the Laboratory of Vision Research (LVR) , NEI, we have continued to investigate the nature of the deficiency in the β27-crystallin in the Philly mouse cataract. New evidence indicates a polyadenylation deficiency in crystallin mRNA.

The group headed by Dr. Toshimichi Shinohara has progressed greatly this year. An important accomplishment has been their establishment of a λ phage gt 11 cDNA library from the human eye which provides a rich source of gene products for the visual system. They have isolated and sequenced a rhodopsin cDNA from this library. With this cDNA, this group has isolated four unique genomic DNA fragments which contain opsin-like sequences. One of these fragments contains the rhodopsin gene. The other three DNA fragments appear to contain opsin genes for the color photopigments, although this remains to be established. These experiments will ultimately lead to a detailed description of the human photopigments at the protein and gene level, and allow investigation of the molecular basis of color blindness and possibly other retinal disorders.

The group headed by Dr. Gabriel Vogeli has also made major advances in their investigations on the collagen genes. Chief among these advances is the isolation and sequence of a murine type IV collagen cDNA. This has led to the isolation of type IV collagen genes from mice, humans, and chickens. The murine type IV gene was shown to have many introns, as does the chicken α2 type I collagen gene. This group has also isolated cDNA clones for α1 and α2 type I collagen, type 2 collagen, and sea urchin basement membrane collagen. These newly acquired cDNAs permit studies on the developmental regulation of the different collagens throughout the eye and on evolutionary aspects of these key extracellular components. In addition, they allow exploration of possible disorders in collagen gene expression during disease. In this connection, collaborative studies with Drs. Peter Kador, Jane Miller and Jin Kinoshita (LVR, NEI) on type IV collagen synthesis in a diabetic mouse model have been initiated.

The group headed by Dr. Peggy Zelenka continues to provide important insight into the biochemical mechanisms regulating lens cell growth and differentiation. An important discovery of this group is that indomethacin (an agent which blocks arachidonic acid metabolism) stimulates the effects of various growth factors (epidermal growth factor, insulin, fetal calf serum, and retinoblastmoma derived growth factor) on cultured Nakano mouse lens epithelial cells. This suggests that some arachadonic acid metabolite may be a negative regulator of lens cell growth. In addition, they have found a novel arachidonic acid metabolite, possibly related to the prostaglandins, in the cultured lens cells treated with these growth factors. Finally, this group is describing further their important finding reported last year relating embryonic chicken lens cell growth with phosphotidylinositol (PI) turnover. They have now established that the rate of PI turnover is proportional to the stimulation of DNA synthesis and cell division in the Nakano mouse lens cells cultured in the presence of growth factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00126-03 LMDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Crystallin Genes: Structure, Organization, Expression, and Evolution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joram Piatigorsky	Ph.D.	Chief	LMDB, NEI
Others:	Ana B. Chepelinsky	Ph.D.	Expert	LMDB, NEI
	J. Fielding Hejtmancik	M.D., Ph.D.	Medical Staff Fellow	LMDB, NEI
	John M. Nickerson	Ph.D.	Staff Fellow	LMDB, NEI
	Teresa Borras	Ph.D.	Staff Fellow	LMDB, NEI
	Barbara Norman	B.S.	Chemist	LMDB, NEI
	James W. Hawkins	Ph.D.	Fogarty Fellow	LMDB, NEI
	Gokul Das	Ph.D.	Visiting Associate	LMDB, NEI

COOPERATING UNITS *(if any)*

See next page.

LAB/BRANCH
Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.8	9.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing to examine the structure, organization, expression, and evolution of the crystallin genes of the eye lens. The single αA-crystallin gene has been cloned from chickens and has a generally similar exon-intron structure to that of the αA-crystallin gene from mice; the human αA-crystallin gene has also been cloned and partially characterized. Hybridization studies have demonstrated differential temporal and spatial regulation of the δ- and various members of the β-crystallin gene families in the developing chicken lens. The β35-crystallin cDNA and gene are being extensively characterized, since this is the most fiber cell specific crystallin in the chicken lens. Five γ-crystallin cDNAs and one γ-crystallin gene from the mouse have been sequenced. The results showed that the γ-crystallin protein domains are encoded in separate exons, and that the greatest diversity among the γ-crystallins is in the third structural motif of the polypeptides. The promoter of the mouse αA-crystallin gene has been used to drive the bacterial chloramphenicol acetyl transferase (CAT) gene in primary explants of transfected embryonic chicken lens epithelia; initial tests indicate that at least 400 base pairs of 5' flanking sequences contribute to the tissue-specific expression of this gene. The promoter regions of the two linked δ-crystallin genes have been sequenced, transcribed in vitro and used to express the CAT gene in transfected lens cells. All experiments suggest appreciable functional differences between these two similar genes. Characterization of the new turtle crystallin (τ-crystallin) raises the possibility that it is related to δ-crystallin and that it represents the origins of this specialized avian and reptilian lens protein.

Additional Personnel Engaged on Project:

Mark A. Thompson	Ph.D.	Staff Fellow	LMDB, NEI
Aida Wakil	B.S.	Chemist	LMDB, NEI
Eric F. Wawrousek	Ph.D.	Staff Fellow	LMDB, NEI
David McDevitt	Ph.D.	Guest Worker	LMDB, NEI
George Inana	M.D.,Ph.D.	Medical Officer	LOP, NEI
Deborah Carper	B.S.	Biologist	LVR, NEI

Cooperating Units:

Martin Breitman	Ph.D.	Hospital for Sick Children Toronto, Canada
Lap-Chee Tsui	Ph.D.	Hospital for Sick Children Toronto, Canada
R. J. M. Gold	M.D.	University of Toronto Toronto, Canada
Joseph Horwitz	Ph.D.	UCLA, Los Angeles, California
Leah A. Williams	Ph.D.	University of West Virginia Morgantown, West Virginia
David C. Beebe	Ph.D.	Uniformed Services University of the Health Sciences Bethesda, Maryland
Robert Church	Ph.D.	Albany Medical College Albany, New York

Objectives: The objective of this project is to understand the structure, organization, expression, and evolution of the gene families encoding the lens crystallins. Particular attention is given to the regulation of crystallin gene expression during lens development and, when possible, to defects in gene function during cataractogenesis.

Methods Employed: Conventional methods for analysis of proteins and nucleic acids are used. These include polyacrylamide gel electrophoresis, isoelectric focussing, protein fingerprinting, RNA and DNA isolation, molecular hybridization, cell-free synthesis, molecular cloning, DNA sequencing, and electron microscopy. Chicken, mice, and turtles are used as experimental animals.

Major Findings:

1. τ-Crystallin, a new turtle lens crystallin which we reported briefly several years ago, has been purified and extensively characterized in collaboration with Drs. J. Horwitz at UCLA Medical School and L. A. Williams at University of West Virginia. Although antigenically distinct from the other crystallins, the α-helical content, amino acid composition, and native and subunit molecular weights suggest that this protein may be related to δ-crystallin. Moreover, since τ-crystallin cross-reacts immunologically with a novel crystallin discovered recently in the lamprey, it may represent the first clue to the origin of δ-crystallin.

2. Five γ-crystallin cDNAs of the mouse lens have been sequenced in collaboration with Drs. Martin Breitman and Lap-Chee Tsui at the Hospital for Sick Children, Toronto, Canada. The results show that these closely related polypeptides are very similar in structure. However, most of the mouse γ-crystallin polypeptides differ from calf γII-crystallin by the absence of one amino acid residue in the peptide connecting the two domains of the protein. In addition, the mouse γ-crystallin polypeptides are most divergent in the third structural motif. Mouse γ-crystallin genes have also been isolated and shown to contain 3 exons as in the rat. The major intron separates the exons encoding the two protein domains. Unlike the β-crystallin genes, the two structural motifs in each domain are encoded within the same exon.

3. Hybridization experiments using a mouse γ-crystallin cDNA suggest that the chicken lacks γ-crystallin genes or pseudogenes. This makes embryonic chicken lens epithelial cells potentially useful recipients for investigating the expression of cloned γ-crystallin genes. Such experiments are in progress.

4. The 5' regions of both, linked δ-crystallin genes have been identified and sequenced. δ1, situated 5' to δ2, appears to be much more active than δ2. For example, all δ-crystallin cDNAs examined by us and others have been derived from δ1. The 5' flanking sequences of δ1 have a viral core-enhancer-like structure, a CCAAT box, and a TATA box; by contrast, the 5' flanking sequences of δ2 lack the viral core-enhancer-like structures and CCAAT box. The two δ-crystallin genes appear otherwise to be very similar, despite their differential activity. Interestingly, intron 1 of both genes is homologous to the immunoglobulin heavy-chain switch region of the mouse. The meaning of this is not known.

5. Considerable advance has been made in sequencing the δ-crystallin gene locus in chickens. Approximately 10 kilobases of sequence have been obtained, although there have not yet been completely linked into contiguous segments.

6. Experiments testing the in vivo function of crystallin promoters have been initiated. Promoter sequences of the mouse αA-crystallin gene and of the chicken δ-crystallin genes have been fused with the bacterial chloramphenicol acetyl transferase (CAT) gene using the pSVO vector. This construct has been used in transient expression studies. Primary embryonic chicken lens epithelial explants were transfected and assayed for CAT activity 72 hours later. So far, the mouse αA-crystallin promoter has been more effective in promoting CAT activity than the chicken δ-crystallin promoters, even though chicken lens cells have been used as recipients of the cloned genes. Greater activity was obtained when 400 basepairs (bp) of 5' flanking sequence were used than when 85 bp of 5' flanking sequence were employed. The mouse αA-crystallin gene promoter was not effective in non-lens cells, and thus displayed tissue-specificity.

7. In vitro transcriptional studies have been initiated using the two chicken δ-crystallin gene promoters. These have been subcloned and transcribed in a Hela cell-free extract. The δ1 promoter was at least four times more active in this system than the δ2 promoter. S1 mapping experiments are in progress to identify the initiation site for transcription of the two δ-crystallin promoters in the Hela cell extract.

8. Last year we constructed four different chicken β-crystallin cDNAs. Southern-blot hybridization and gene isolations showed that these were derived from different genes. We have almost completed the sequence of the cDNA encoding the largest β-crystallin polypeptide (35K). In addition, the β35 gene has been isolated, partially sequenced and its promoter identified by in vitro transcription in a Hela cell extract. Particular attention is being devoted to the β35 gene, since we showed that its expression is stringently correlated with fiber cell differentiation. The different β-crystallin mRNAs have been quantified by cDNA hybridization in the chicken lens. Each displayed a characteristic temporal and spatial pattern in the developing lens. δ-Crystallin mRNA accumulated most rapidly in the embryonic lens, while the β-crystallin mRNAs predominated after hatching. Thus, differential regulation occurs both between and within crystallin gene families.

9. The human and chicken αA-crystallin gene have been isolated and characterized by restriction mapping, partial sequencing and, in the case of the chicken, electron microscopy. As in mice, both humans and chickens appear to have a single αA-crystallin gene. Preliminary data indicate that the intron-exon structure of this gene is similar in the three organisms.

10. In collaborative experiments with Dr. George Inana (LOP, NEI) and Ms. Deborah Carper (LVR, NEI), we are continuing to investigate the basis for the absence of the β27 polypeptide in the Philly mouse hereditary cataract. The present data suggest that this protein may be encoded by the β23 gene which we have cloned previously. Polyadenylation of mRNA appears deficient in the Philly lens: this deficiency appears to be greater with the β23 mRNA than with other crystallin mRNAs.

11. A series of chicken δ-crystallin monoclonal antibodies have been generated in collaborative experiments with Dr. Robert Church (Albany Medical College). These have been used to show that there are at least four sizes of δ-crystallin polypeptides in the embryonic lens (52K, 50K, 48K, 45K). This finding raises interesting questions at the gene level, since strong evidence indicates the existence of only two δ-crystallin genes, and only one of these (δ1) has been unequivocally shown to be active.

Significance to Biomedical Research and the Program of the Institute: The lens crystallins are a family of evolutionarily conserved proteins that are differentially expressed in the developing lens and are responsible for lens transparency. Understanding the structure, function and evolution of these protein families and their genes contribute to our knowledge of embryonic development, eukaryotic gene expression, cell differentiation, molecular evolution, the visual system and disease (in particular, cataract).

Proposed Course: The following studies are in progress or proposed for FY 1985:

1. The entire 30Kb chicken δ-crystallin locus in the chicken genome will be sequenced.
2. The sequence of the chicken β35 cDNA and gene will be completed.
3. The chicken and, possibly, human αA-crystallin gene will be sequenced.

4. The sequence and organization of the β23, β25, and β19/26 cDNAs and genes will be examined.

5. The sequences regulating crystallin gene expression will be characterized further. These experiments involve the construction of numerous expression vectors. Promoter activity will be examined in primary explants of the embryonic chicken lens epithelium.

6. A systematic search for the expression of the chicken δ2 gene will be conducted by using gene specific oligonucleotides synthesized on the basis of our ongoing sequence analysis. These oligonucleotides will be used as probes in dot-blot and Northern-blot hybridization experiments, and if positive, for screening cDNA libraries made from the appropriate source.

7. Expression studies using the Sp6 promoter fused to cloned crystallin cDNAs will be conducted.

8. Transgenic mice will be created using crystallin promoters fused to the bacterial CAT gene.

9. Turtle τ-crystallin will be analyzed further and its cDNA will be cloned.

NEI Research Program: Cataract--The Normal Lens

Publications:

Piatigorsky J, Treton JA, King CR, Nickerson JM, Carper D, Shinohara T, Inana G, Hejtmancik JF, and Norman B: A molecular genetic approach to vision research: Crystallin gene expression in the lens. Ophthal Ped Gen 3:61, 1983.

Piatigorsky J: δ-Crystallin and their nucleic acids. Mol. Cell Biochem 59:33, 1984.

King CR and Piatigorsky J: Alternative splicing of αA-crystallin RNA. Structural and quantitative analysis of the mRNAs for the αA_2-and the αA^{ins}-crystallin polypeptides. J Biol Chem 295:1822, 1984.

Sun, S-T, Tanaka T, Nishio I, Peetermans J, Maizel, JV Jr., Piatigorsky, J: Direct observation of δ-crystallin accumulation by laser light-scattering spectroscopy in the chicken embryo lens. Proc Natl Acad Sci USA 81:785, 1984.

Nickerson JM and Piatigorsky J: Sequence of a complete chicken δ-crystallin cDNA. Proc Natl Acad Sci USA 81:2611, 1984.

Lok S, Tsui L-C, Shinohara T, Piatigorsky J, Gold R, Breitman ML: Analysis of the mouse γ-crystallin gene fammily: Assignment of multiple cDNAs to discrete genomic sequences and characterization of a representative gene. Nucleic Acids Res 12:4517, 1984.

Hawkins JW, Nickerson JM, Sullivan MA, and Piatigorsky J: The chicken δ-crystallin gene family. Two genes of similar structure in close chromosomal approximation. J Biol Chem 259:9821, 1984.

Piatigorsky J, Nickerson JM, King CR, Inana G, Hejtmancik J, Hawkins JW, Borras T, Shinohara T, Wistow G, and Norman B: Crystallin genes: Templates for lens transparency. In Human Cataract Formation, Ciba Foundation Symp. No. 106, Nugent J, editor. Eisevier, Pitman Publishers (in press).

Inana G, Carper D, and Piatigorsky J: Crystallins and cataractogenesis: A molecular genetics approach. In Hereditary and Visual Development, Eight Symp. on Ocular and Visual Development, Hilfer SR and Sheffield JB, editors. Springer-Verlag New York (in press).

Piatigorsky J, Chepelinsky AB, Hejtmancik JF, Borras T, Das GC, Hawkins JW, Zelenka PS, King CR, Beebe DC, and Nickerson, JM: Expression of crystallin gene families in the differentiating eye lens. In Molecular Biology of Development, Cetus-UCLA Symposium, Davidson E and Firtel R, editors. Alan R. Liss, New York, NY (in press).

Treton JA, Jones RE, King CR, and Piatigorsky J: Evidence against γ-crystallin DNA or RNA sequences in the chicken. Exp Eye Res (in press).

Piatigorsky J: Lens crystallins and their gene fammilies. Cell (in press).

Breitman ML, Lok S, Wistow G, Piatigorsky J, Treton JA, Gold RJM, and Tsui L-C: The δ-crystallin family of the mouse lens: Structural and evolutionary relationships. Proc Natl Acad Sci USA (in press).

Garber AT, Winkler C, Shinohara T, King CR, Inana G, Piatigorsky J, and Gold RJM: Selective loss of a family of gene transcripts in a hereditary murine cataract. Science (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00132-03 LMDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Photopigments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Toshimichi Shinohara	Ph.D.	Biologist	LMDB, NEI
Others:	Graeme J. Wistow	Ph.D.	Fogerty Fellow	LMDB, NEI
	James P. Alligood	B.S.	Biologist	LMDB, NEI
	Albine Katial	Ph.D.	Staff Fellow	LMDB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular and Developmental Biology

SECTION
-

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure of the mRNA and genes for photopigments is being examined. The cDNAs made from human eyes were cloned into phage λ gt 11 using EcoR1 linkers and a human cDNA library was established. A human opsin cDNA (1 Kb long) was isolated from the cDNA library by cross-hybridization with a bovine cDNA probe, and identified by DNA sequence determination.

We previously have obtained four different genomic clones from placenta and spleen libraries by cross-hybridization with a bovine opsin cDNA probe. One of the genomic clones was identified as a human opsin gene by cross-hybridization with human opsin cDNA and by restriction enzyme mapping. An X chromosome, Hind III DNA library screening experiment suggested that two of the human genomic clones are on the X chromosome. One of the genes is nearly full-size, the other is about one half of the gene. We do not know whether these genes are pseudogenes or polymorphic alleles. They are being sequenced in order to establish their primary gene structure. Since rhodopsin and color pigments are likely to have related primary structures, it is possible that some of these genes (particularly X chromosome linked genes) are indeed those coding for human color pigments.

This analysis is a first step toward a molecular analysis of color-blindness at the gene level.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The objectives of this project are to understand the structure, organization, evolution, and function of photopigments (rhodopsin and color pigments) and their genes in the retina. Particular attention is given to human genes. Emphasis is given both to the normal and the abnormal retina, particularly to hereditary disorders such as color blindness and retinal degenerations.

Methods Employed: Conventional methods for cDNA cloning and screening and genomic DNA screening are used. These include isolation of mRNA and DNA, cDNA cloning (G-C tailing into pBR322 and EcoR1 linkers into λ gt 11), colony screening on Millipore filters, nick translation, agarose gel electrophoresis, DNA sequencing by the chemical degradation and the dideoxy methods, Southern and Northern blot hybridization, and phage plaque hybridization. Bovine, rat, monkey, and human retina are used for the experiments.

Major Findings:

1. A cDNA library for the human eye has been constructed. This library is a source of many mRNAs from the human eye, and opens the door to genetic studies of the eye at the molecular level.

2. A human opsin cDNA has been obtained from the cDNA library and has been partially sequenced. The size of this cDNA is about 1 kilobase.

3. Four different, distinct human genomic clones have been isolated from human placenta and spleen genomic libraries (gift from P. Leder, Harvard Medical School) by cross-hybridization with bovine opsin cDNA (from Dr. M. Applebury, Purdue University).

4. One of the genomic clones has been unequivocally identified as opsin by cDNA hybridization and restriction enzyme mapping.

5. Two of the genomic clones are associated with the X chromosome.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the molecular mechanism of visual excitation (transduction) is of fundamental importance in eye research. The photopigments present in rod (rhodopsin) and cone cells (color pigments) are responsible for the transduction. Relatively little is known about the photopigments, and nothing is known of any color pigment genes. Recombinant DNA technology provides new and powerful methods for the study of photopigments. Direct analysis of cloned cDNAs and their genes can provide knowledge of protein primary sequences, gene structures, differential gene activity for normal and abnormal photopigments and evolution of vision. Also, specific mutagenesis of recombinant DNAs can provide a means for identifying critical regions for the function of these visually important proteins. A molecular analysis of color-blindness at the gene level is of importance not only from a medical viewpoint but also because

it is a source of interesting mutant genes. Approximately 9% of the male population are color blind, and it is likely that numerous classes of mutations will be associated with this condition.

Proposed Course: The following experiments will be performed or initiated in the next fiscal year:

1. In situ hybridization of identifiable cone cells using opsin cDNA probes.
2. In situ hybridization using hybrid cells in order to map the opsin genes to their chromosomes.
3. Expression of cloned photopigment cDNAs in bacteria and spectroscopic analysis of the product when combined with retinal.
4. Identification of abnormal color-pigment genes from color-blind subjects by restriction enzyme analysis and Southern blot hybridization. Abnormal genes may give abnormal restriction maps. cDNAs can then be assigned their respective genes by hybridization to restriction fragments of the defective genes. Once defective genes responsible for color-blindness have been isolated, they will be sequenced and analyzed in detail.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction.

Publications:

Lok S, Tsui L, Shinohara T, Piatigorsky J, Gold R, and Breitman J: Analysis of the mouse γ-crystallin gene family: Assignment of multiple cDNAs to discrete genomic sequences and characterization of a representative gene. Nucl Acids Res 12:4517, 1984.

Piatigorsky J, Treton J, King C, Nickerson J, Carper D, Shinohara T, Inana G, Hejtmancik F, and Norman B: A molecular genetic approach to vision research: crystallin gene expression in the lens. Opthal Ped and Genetics 3:61, 1983.

Piatigorsky J, Nickerson J, King C, Inana G, Hejtmancik F, Hawkins J, Borras T, Shinohara T, Wistow G, and Norman B: Crystallin genes: Templates for lens transparancy. In Human Cataract Formation, Ciba Foundation Symp. No. 106, Nugent J, editor. Elsevier, Pitman Publishers (in press).

Carper D, Russell P, Shinohara T, and Kinoshita JH: Differential synthesis of rat lens proteins during development. Exp Eye Res (in press).

Garber AT, Winkler C, Shinohara T, King CR, Inana G, Piatigorsky J, and Gold RJM: Selective loss of a family of gene transcripts in a hereditary murine cataract. Science (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00128-03 LMDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Collagen Genes: cDNA Clones and Genomic Clones for Types I, II, and IV Collagen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gabriel Vogeli	Ph.D.	Senior Staff Fellow	LMDB, NEI
Others:	Pravendra Nath	Ph.D.	Fogarty Fellow	LMDB, NEI
	Elizabeth Horn	B.S.	Biological Help	LMDB, NEI
	Cynthia Jaworski	M.S.	Graduate Student	LMDB, NEI

COOPERATING UNITS *(if any)* Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, NIH (M. Young); Laboratory of Pathology, National Cancer Institute, NIH (M. Sobel); Division of Biochemistry and Biophysics, National Center for Drugs and Biologics, FDA (G. Zon).

LAB/BRANCH
Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNA and genomic clones for different types of collagens have been isolated and characterized. Type IV collagen is the main collagen of the lens capsule and is found in all basement membranes. A set of synthetic oligonucleotides (CCCATA/GAANCCT/CTC) was derived from the published amino acid sequence (Glu-Gly-Phe-Met-Gly) for alpha 1 Type IV collagen. This set of oligonucleotides was labelled with P^{32} and hybridized to the 100,000 colonies of a mouse cDNA library. One colony was isolated that had an insert coding for alpha 1 Type IV collagen. This colony (pCIV-1-225) codes for 270 amino acids from the helical portion of the Type IV collagen. There are four interruptions in the Gly-X-Y repeats. Northern analysis shows that the mRNA for the alpha 1 Type IV collagen is 7,400 bases long, suggesting that it contains a large untranslated region. With the help of the cDNA clone, we isolated genomic clones for the alpha 1 Type IV collagen from mouse, human, and chicken. The mouse genomic clone shows a gene structure that is as complex as the gene structure of alpha 2 Type I collagen gene from the chicken.

Type I collagen cDNA clones for both chains (alpha 1 and alpha 2) from the mouse have been isolated and characterized. Type I collagen synthesis in the cornea will be studied with these molecular probes. Type II collagen cDNA clones and genomic clones from the chicken have been isolated and characterized. Type II collagen synthesis for the vitreous body will be analyzed. Sea urchin collagen clones related to the basement membrane collagen of the mouse have been isolated and are being characterized.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The object of this work is to analyze, characterize, and understand the structure, regulation, and evolution of the collagen gene family. The vertebrate eye contains abundant extracellular matrixes: the basement membranes of the lens and the capillaries, the stroma of the cornea, the connective tissues of the sclera, and the vitreous body. Molecular probes necessary for the study of basement membrane synthesis, of vitreous body synthesis, and of cornea and connective tissue synthesis are being isolated and characterized. These probes will be used to study eye development and to analyze pathological changes in eye tissues.

Methods Employed: Standard techniques of recombinant DNA technology are being used to isolate and characterize cDNA and genomic clones. This includes agarose and acrylamide gel electrophoresis, hybridization, RNA and DNA isolation, electron microscopy, and sequence analysis. Mouse and chicken are used as experimental animals.

Major Findings:

1. The recombinant plasmid pCIV-1-225 has a mouse cDNA insert that codes for alpha 1 Type IV collagen.
2. The complete insert of pCIV-1-225 has been sequenced. It contains 820 basepairs coding for a portion of the triple helical region of the alpha 1 Type IV collagen. It is the first nucleotide sequence available from a Type IV collagen cDNA clone.
3. The triple helical structure is interrupted four times by amino acid sequences that cannot form the triple helix. One of these interruptions has the same sequence as an interruption found in the type 9 collagen.
4. Two amino acid stretches are repeated within our sequence. One stretch is 10 amino acids long, the other is 7 amino acids long.
5. The mRNA coding for alpha 1 Type IV collagen is 7,400 bases long and, therefore, will contain large noncoding sequences.
6. pCI-2-345 is an alpha 2 Type I cDNA clone from mouse. It has been partially sequenced.
7. pCI-1-27430 is an alpha 1 Type I collagen cDNA clone from the mouse. It has been partially sequenced.
8. A prospective alpha 2 Type IV collagen cDNA clone (pCIV-2-176) is being sequenced. It contains an insert of around 1.8 Kb.
9. The structure of a genomic alpha 1 Type IV collagen clone has been revealed by D-looping. The gene structure is as complex as the gene structure for the alpha 2 Type I collagen from chicken.
10. Genomic clones from human and from chicken have been isolated.
11. Several basement membrane related genomic collagen clones have been isolated from sea urchins. They are being characterized now.

Significnace to Biomedical Research and the Program of the Institute: The physical shape of the eye is determined by the synthesis of different types of extracellular matrices during development. Cartilages, bones, and other connective tissues containing mainly Type I collagen determine the overall form

of the eye. The correct spatial arrangement of Type I collagen in the cornea is responsible for its transparency and for the major change in the refractive index.

The basement membrane of the lens with its Type IV collagen provides the physical form of the lens and allows the lens to change shape during focusing. Type IV collagen is the collagen of the capillary basement membranes of the retina. In many of the pathogenic events affecting the eye, extracellular matrix components are involved. Diabetes, by affecting the basement membranes in blood vessels, can be destructive to the overall well-being of the retina. Type II collagen in the vitreous body maintains the optical properties of this extracellular matrix. Thus, an understanding of the events at the molcular level during the biosynthesis of extracellular matrix components will help to understand the course of many disease processes in the eye.

Proposed Course:

1. We will characterize the genomic clones for the alpha 1 Type IV collagen from mouse, human, and chicken. This will provide information concerning the evolution of Type IV collagen. It will also provide molecular probes to study the Type IV collagen gene in humans and in chickens.
2. We will "walk along" the gene and along the cDNA towards the 5' end. The "cDNA walking" and the "genomic walking" will complement each other. The goal is to analyze regulatory elements (promoter) located at the 5' side of the gene.
3. By cross-hybridization at low stringency, we have isolated prospective alpha 2 Type IV collagen clones. We are sequencing and analyzing these clones. Since the molecular structure of the Type IV collagen is characterized by its interrupted triple helix, the knowledge of the alpha 2 subunit will help to understand the biochemistry of Type IV collagen.
4. We will isolate and characterize genomic clones for the alpha 2 Type IV collagen.
5. In collaboration with Drs. Peter Kador, Jane Miller and Jin Kinoshita (Laboratory of Vision Research, NEI) we started to analyze Type IV collagen synthesis in a diabetic mouse model.
6. In collaboration with Drs. Malabi Venkatesanand and Robert Simpson (Laboratory of Cellular and Developmental Biology, NIADDK) we are isolating genomic clones for basement membrane collagen from sea urchins. Sea urchins are excellent model systems for the study of early development.

NEI Research Program: Cataract--Diabetes

Publications:

Young MF, Vogeli G, Nunez AM, Fernandez MP, Sullivan M, Sobel ME: Isolation of cDNA and genomic DNA clones encoding type II collagen. Nucleic Acid Res 12: 4207, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00127-08 LMDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Peggy Zelenka	Ph.D.	Geneticist	LMDB, NEI
Others:	Ngoc-Diep Vu	Ph.D.	Staff Fellow	LMDB, NEI
	Luke Pallansch	Ph.D.	Staff Fellow	LMDB, NEI

COOPERATING UNITS *(if any)*
Beltsville Agricultural Research Center, Beltsville, MD (A. Ferretti)

LAB/BRANCH
Laboratory of Molecular and Developmental Biology

SECTION

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to determine whether the regulation of lens fiber differentiation and maturation is associated with alterations in the plasma membrane. The composition, biosynthesis, and metabolism of lens lipids have been investigated using embryonic and adult chicken lenses, and cultured lens epithelial cells derived from the Nakano mouse. The rate of degradation of the membrane phospholipid, phosphatidylinositol, has been shown to be tightly coupled to the rate of lens epithelial cell division and to cease when the epithelial cells differentiate to form lens fibers. Since phosphatidylinositol is rich in arachidonic acid, a precursor of prostaglandins and leukotrienes, the metabolities of arachidonic acid produced by lens epithelial cells are being characterized in an effort to understand the physiological role of phosphatidylinositol degradation. Alterations in phosphatidylinositol metabolism and in the production of arachidonic acid metabolities are being correlated with the action of growth factors in regulating cell division and differentiation.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The objectives of this project are to understand the composition and biosynthesis of lens lipids and to determine whether alterations in the lipid components of lens membranes play a role in the regulation of cell growth and differentiation.

Methods Employed: In vitro studies of lens phospholipid metabolism employ explants of lens epithelia from embryonic chickens of various ages or a clonal cell line of Nakano mouse lens epithelial cells developed by Paul Russell (Laboratory of Vision Research, NEI). Cells are grown by conventional culture techniques. Explants can be maintained in an epithelial cell state for several hours or forced to differentiate into lens fibers by manipulating the culture medium. Metabolism of phospholipids and arachidonic acid in explants or cultured cells is investgated by adding appropriate radioactive precursors to the culture medium. Growth factors or drugs are added to the medium to determine the relationship between phospholipids and arachidonic acid metabolities, on the one hand, and cell growth and differentiation on the other.

Cell cycle parameters and the proliferative index of lens epithelial cells at various developmental stages are determined by continuous, in ovo labeling of cells with ^{3}H-thymidine. The fraction of cells labeled at different times is determined by autoradiography, and the labeling kinetics are analysed using computer curve-fitting techniques.

Major Findings:

1. PI degradation and cell cycle regulation. Previous results had indicated that in developing embryonic chicken lens epithelial cells the rate of PI degradation is tightly coupled with the rate at which the cells are labeled during a brief exposure to ^{3}H-thymidine. However, a number of parameters determine the rate of ^{3}H-thymidine incorporation in a pulse-labeling experiment. To separate these parameters we have undertaken a series of continuous-labeling experiments to determine cell cycle parameters and the proliferative index of central lens epithelial cells as a function of developmental age. Preliminary analysis of the data indicates that the length of G_1 increases during development and the proliferative index falls. Interestingly, both of these factors seem to correlate with the rate of PI degradation.

2. PI degradation and the action of growth factors. The correlation between the rate of PI degradation and cell division has been extended to cultured, Nakano mouse lens epithelial cells stimulated to divide by the addition of various growth factors to the medium. All growth factors known to be mitogenic for these cells also stimulated PI degradation. Moreover, the rate of cell division, as judged by the incorporation of ^{3}H-thymidine, was in each case proportional to the rate of PI degradation stimulated by the agent. Indomethacin, an inhibitor of prostaglandin biosynthesis, further stimulated cell division when added in conjunction with a growth factor, but did not further stimulate PI degradation. This finding implies that the rate of PI

degradation is not a consequence of the rate of cell division. Thus, the correlation between PI degradation and cell growth may be attributed to an effect of PI metabolism on the regulation of the cell cycle. Furthermore, the stimulatory effect of indomethacin of ^{3}H-thymidine incorporation suggests that a product of the cyclo-oxygenase pathway of arachidonic acid metabolism may be a negative regulator of lens epithelial cell growth.

3. Arachidonic acid metabolites. The finding that indomethacin enhances the effect of growth factors on cultured lens epithelial cells has made analysis of the arachidonic acid metabolites produced by cultured lens epithelial cells particularly important. We have tentatively identified four minor products of arachidonic acid metabolism in cultured, Nakano lens epithelial cells on the basis of their drug sensitivities and chromatographic behavior; two lipoxygenase products, one of which comigrates with 15-hydroxy eicosatetraenoic acid (15-HETE) and two cyclo-oxygenase products, PGE_2 and $PGF_{2\alpha}$. However, the major metabolite of arachidonic acid in this cell line is an as yet unidentified product which has the drug sensitivity and UV spectrum of a prostaglandin, yet does not comigrate with any of the known parent compounds by HPLC. Further investigation of the structure of this product is being carried out in collaboration with Dr. Aldo Ferretti, Beltsville Agricultural Research Center, Beltsville, MD.

Significance to Biomedical Research and the Program of the Institute: The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition, they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. These results should have broad application in understanding normal lens differentiation and morphogenesis and in attempts to establish etiologies for several types of cataract.

Proposed course: The following studies are in progress or proposed for FY 1984:

1. Further analysis of cell cycle kinetics of central lens epithelial cells in developing chicken embryos.
2. Determination of the structure and possible biological activity of the major arachidonic acid metabolite of cultured, Nakano mouse lens epithelial cells.
3. Biochemical studies of the mechanism of action of growth factors in lens cells, including investigation of the role of phosphatidylinositol phosphate and phosphatidylinositol-bis-phosphate.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka PS and Vu ND: Phosphatidylinositol degradation is directly proportional to cell division in embryonic chicken lens epithelia. In The Chilton Conference on Inositol and Phosphoinosiutides, Bleasdale J, Eichberg J, and Hansen G, editors. New York, Humana Press (in press).

Zelenka PS and Vu ND: Correlation between phosphatidylinositol degradation and cell division in embryonic chicken lens epithelia. Develop Biol (in press).

LABORATORY OF OPHTHALMIC PATHOLOGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00145-02 LOP

PERIOD COVERED: October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Effects of Aging and Nutrition on the Retina and Retinal Pigment Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Martin L. Katz	Ph.D.	Staff Fellow	LOP, NEI
Others:	W. Gerald Robison, Jr.	Ph.D.	Chief, Section on Experimental Anatomy	LOP, NEI

COOPERATING UNITS *(if any)*: Eye Research Foundation, University of Missouri (G. Eldred).

LAB/BRANCH: Laboratory of Ophthalmic Pathology

SECTION: Section on Experimental Anatomy

INSTITUTE AND LOCATION: NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use stendard unreduced type. Do not exceed the space provided.)*

Age-related changes in the retina and retinal pigment epithelium (RPE) have been characterized using morphological and biochemical techniques. One of the major age-related changes in the RPE is the progressive accumulation of lipofuscin, or age-pigment. This pigment is autofluorescent, and we have succeeded in extracting and separating a number of fluorophores from both rat and human RPE lipofuscin. The fluorophores from both species are remarkably similar, suggesting that the rat may serve as a good model for studying the factors responsible for lipofuscin accumulation in the human RPE. Because of the importance of RPE plasma membrane functions in maintaining the health of the neural retina, the basal infoldings of the pigmented rat RPE were examined for age-related alterations. Quantitative morphometric analysis revealed that during senescence the amount of basal plasma membrane per unit RPE cell length increased substantially, the regional distribution of the basal infoldings along the RPE became more irregular, and the average depth of penetration of the basal infoldings into the RPE increased dramatically. These studies have brought us closer to an understanding of the factors responsible for age-related retinopathies.

Project Description

Additional Personnel Engaged on Project:

Anne Groome	Histologist	LOP, NEI

Objectives: To study age-related changes in the retinal pigment epithelium (RPE) and determine the mechanisms underlying these changes. Particular attention has been given to determining the mechanisms of lipofuscin formation in the RPE and to examining the RPE for age-related changes which might have significance for the health of the neural retina.

Methods Employed: Eyes from Fisher 344 albino rats, as well as normal human eyes from an eye bank, were used for lipofuscin fluorophore characterization. Fluorophores were extracted with organic solvents and separated by high performance thin layer chromatography. Morphometric analyses on the RPE basal infoldings from eyes of pigmented rats were performed on electron micrographs using a new computer-based morphometric analysis system.

Major Findings:

1. Lipofuscin fluorophores from both human and rat RPE have been extracted and separated into a number of components. The pattern of fluorophores from rat and human RPE lipofuscin are quite similar.

2. The morphology of the RPE basal infoldings was found to undergo a dramatic alteration during senescence in pigmented rats. The amount of basal plasma membrane per unit RPE length increased about 60% between 4 and 32 months of age. The regional distribution of the basal infoldings along the RPE became quite irregular during aging. In addition, the average depth of penetration of the basal infoldings into the RPE increased dramatically.

3. These changes in RPE morphology were accompanied by a pronounced thickening of the RPE basal lamina. In young rats, the basal lamina appeared as a thin line of electron-opaque material parallel to the bases of the RPE cells. In the older rats, this layer of material had increased dramatically in thickness, and often extended up into the RPE basal infoldings. Embedded in this basal lamina material of older rats were some unique banded structures not seen in the younger animals.

Significance to Biomedical Research and the Program of the Institute: In the U.S. today, senile changes of the retina are among the most prevalent causes of serious visual impairment. Several reports indicate that the incidence of senile macular degeneration (SMD) reaches about 30% in the population between 75 and 85 years of age. This problem is likely to increase dramatically in the current decade, as more people survive to older ages. Through our studies, we hope to gain an understanding of the fundamental processes underlying age changes in the retina. Ultimately, we hope to develop a rational approach for preventing the development of degenerative retinal changes in the elderly.

Proposed Course: We have previously characterized a number of factors which influence the rate of lipofuscin accumulation in the rat RPE. Among these factors are dietary levels of vitamins E and A. Now that we are able to

separate lipofuscin into a number of distinct components, we plan to do experiments to determine which of these components are influenced by vitamin E and A intake in rats. We also plan to purify and characterize the various fluorophores which make up RPE lipofuscin. These experiments will allow us to gain a better understanding of the mechanisms underlying the age-related accumulation of lipofuscin in the RPE.

We have now established a colony of pigmented rats of various ages. We plan to use these rats to further characterize age-related changes in the retina and RPE. Among the features we will examine are the retinal capillaries, cell numbers in the retina, rates of rod outer segment turnover, and vitamin A exchange between the retina and RPE.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium.

Publications:

Katz ML and Robison WG: Lipofuscin response to the "aging-reversal" drug centrophenoxine in rat retinal pigment epithelium and frontal cortex. J Gerontol 38:525, 1983.

Katz ML and Robison WG: Age-related changes in the retinal pigment epithelium of pigmented rats. Exp Eye Res 38:137, 1984.

Katz ML, Robison WG, Herrmann RK, Groome AB, and Bieri, J.G.: Lipofuscin accumulation resulting from senescence and vitamin E deficiency: Spectral properties and tissue distribution. Mech Age Dev 25:149, 1984.

Katz ML, Robison WG, and Dratz EA: Potential role of autoxidation in age changes of the retina and retinal pigment epithelium of the eye. In Free Radicals in Molecular Biology and Aging. Armstrong D, Sohal R, Cutler R, and Slater T (editors). New York, Raven Press (in press).

Katz ML and Robison WG: Antioxidant deficiencies as models for studying the role of autoxidation in senescence. In Free Radicals, Aging, and Degenerative Diseases. Johnson JE (editor). New York, Alan R. Liss (in press).

Katz ML and Robison WG: Age-related and vitamin E-related lipofuscin in ocular and other tissues. Invest Ophthalmol Vis Sci 25(suppl): 208, 1984.

Katz ML and Robison WG: Age-related alterations in retinal pigment epithelium basal plasma membrane morphology. AGE (in press).

Nagata M, Katz ML, and Robison WG: Retinal capillary basal lamina thickening during senescence. AGE (in press).

Katz ML and Robison WG: Senescence and the retinal pigment epithelium: Alterations in basal plasma membrane morphology. Mech Age Dev (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 EY 00149-11 LOP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ultrastructure and Function of the Pigmented and Nonpigmented Cells of the Eye

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Gerald Robison, Jr.	Ph.D	Chief, Section on Experimental Anatomy	LOP, NEI
Others:	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LOP, NEI
	Martin L. Katz	Ph.D.	Staff Fellow	LOP, NEI
	Roland K. Herrmann	M.D.	Visiting Fellow	LOP, NEI

COOPERATING UNITS *(if any)*

Laboratory of Cellular and Developmental Biology, NIADDK, NIH, (J. Bieri).

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Experimental Anatomy

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	2.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Capillary basement membrane (BM) thickening characteristic of diabetes has been found in galactosemic rats and evidence for the involvement of aldose reductase (AR) in the process has been documented. In order to test the possible role of this enzyme, two structurally unrelated but similarly potent inhibitors of AR were utilized: sorbinil (Pfizer) and tolrestat (Ayerst). Weanling male SD rats were separated into three groups and fed for 28, 32, and 44 wks: (1) Lab feed (NIH-07); (2) lab feed with galactose (50%); or (3) lab feed with galactose and AR inhibitor (.03% or .04%). Electron micrographs (x25,000) of capillaries from the retina were examined for BM thickness using quantitative computer planimetry on at least 10 capillaries from each of 3-10 rats per dietary group. The BM, cell, and vessel perimeters were traced over graphics tablets and the areas determined with either the NIH DEC 10 or an IBM personal computer using the appropriate digitizing programs. The results were analyzed with the NIH PROPHET computer system. The basement membranes in ocular blood vessels of rats on galactose chow were thicker than those of rats on either control feed or feed containing an AR inhibitor. Up to a two-fold increase in BM thickness occurred in retinal capillaries at 44 weeks. This thickening of BM must be related to AR activity since it did not occur in galactosemic rats (ca. 200 µg/dl) treated with either sorbinil or tolrestat which are very dissimilar inhibitors. Galactosemic rats should be useful models for studying BM-related complications of diabetes and their possible prevention by AR inhibitors.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To determine if galactosemic rats could serve as useful models for diabetes and if some of the diabetic-like tissue changes could be prevented by inhibitors of aldose reductase.

Methods Employed: Capillary basement membrane thickening was used as a marker of a possible diabetic-like condition, and aldose reductase inhibitors were used to prevent this condition and demonstrate possible involvement of aldose reductase. Male Sprague-Dawley rats were separated into three groups at weaning and fed a control diet of NIH-07 laboratory feed (Zeigler Bros.), a galactose diet having a 1:1 ratio by weight of galactose to NIH-07, or a galactose and aldose reductase inhibitor diet consisting of the galactose diet with .03% or .04% sorbinil (Pfizer) or tolrestat (Ayerst) added per kg diet. After 28, 32, and 44 weeks, the right eyes of three or four rats from each group were enucleated and fixed by immersion in 2.5% glutaraldehyde buffered to pH 7.2 with 50 mM sodium cacodylate. Narrow portions (0.2 x 1.0 mm) oriented radially from near the optic nerve were taken from the superior temporal sector of the central retina and postfixed in OsO_4. This was followed by standard preparation for electron microscopy. To minimize variability for the quantitative analyses, the ultrastructural examination was limited to the outer plexiform layer of the retina. Micrographs were taken of all the capillaries encountered in that region in at least four sections representing slightly different areas of the superior temporal sector of the central retina.

Major Findings: A two-fold thickening of capillary basement membranes of rat retinas resulting from dietary galactose was prevented by sorbinil, and by tolrestat, two unrelated inhibitors of aldose reductase. Since the basement membrane thickening was ultrastructurally similar to that typical of diabetic retinopathy, it may indicate changes in vessel permeability and susceptibility to hemorrahage. Galactosemic rats should be useful models for studying basement membrane-related complications of diabetes and for examining the potential biochemical regulation of basement membrane synthesis by aldose reductase inhibitors.

Significance to Biomedical Research and the Program of the Institute: Aldose reductase, which has been implicated in sugar cataracts, certain corneal healing defects, and peripheral neuropathy of diabetic and galactosemic animals, now appears to be involved in other diabetic-like pathologies. While the normal physiological role of this enzyme in most tissues remains unknown, under the conditions of high plasma sugar concentrations encountered in diabetes and galactosemia, aldose reductase converts these sugars to their respective sugar alcohols (polyols). These polyols are not readily metabolized, nor do they penetrate cell membranes easily. Thus, once formed at significant rates, they may accumulate to very high levels in cells, leading to hypertonicity, alteration of ion permeability, and eventual cell death with consequent tissue changes such as cataract formation. Treatment of diabetic or galactosemic rats with potent aldose reductase inhibitors, such as sorbinil, decreases the accumulation of polyols, which in turn appears to prevent the formation of cataracts in lenses, defective healing in scraped corneas, and decreased conduction velocity in motor nerves.

Recently, aldose reductase was implicated in the formation of the thicker, yet apparently more porous, basement membranes found throughout the vasculature of chronic diabetics. Such vascular changes could contribute to several of the complications of diabetes, including retinopathy, nephropathy, and peripheral microangiopathy. If aldose reductase is indeed involved in such complications of diabetes, then similar diabetic-like pathological conditions should occur in the galactosemic state. The present study was initiated to determine whether early microangiopathic changes occur in the retinal capillaries of galactosemic rats and, if so, whether they can be prevented by oral administration of aldose reductase inhibitors.

Diet-induced galactosemia resulted in a time-related thickening of basement membranes in retinal capillaries that could be prevented by the aldose reductase inhibitors sorbinil and tolrestat. This suggests that aldose reductase may be involved in the thickening of basement membranes.

Similar studies on the basement membranes in kidneys and other tissues should reveal how useful galactosemia might be as a model for diabetes and to what extent sorbinil and tolrestat might be effective in retarding or preventing other complications of diabetes.

Proposed Course: We plan to examine other ocular tissues for possible basement membrane thickening in galactosemic rats and for prevention of thickening with aldose reductase inhibitors. Another important investigation would be to determine if thickening of basement membrane could be reversed by aldose reductase inhibitors.

NEI Research Program: Retinal and Choroidal Diseases-Retinal Pigment Epithelium.

Publications:

Katz ML, and Robison WG Jr: Lipofuscin response to the "aging-reversal" drug centrophenoxine in rat retinal pigment epithelium and frontal cortex. J Gerontol 38:525, 1983.

Robison WG Jr, Kador PF, and Kinoshita JH: Retinal capillaries: basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. Science 221:1177, 1983.

Katz ML, and Robison WG Jr: Age-related changes in the retinal pigment epithelium of pigmented rats. Exp Eye Res 38:137, 1984.

Katz ML, Robison WG Jr, Herrmann PK, Groome AH, and Bieri J.G.: Lipofuscin accumulation resulting from senescence and vitamin E deficiency: spectral properties and tissue distribution. Mec Age Dev 25:149, 1984.

Herrmann RK, Robison WG Jr, and Bieri JG: Deficiencies of vitamins E and A in the rat: lipofuscin accumulation in the choroid. Invest Ophthalmol Vis Sci 25:429. 1984.

Cogan DG, Kinoshita JH, Kador PF, Robison WG Jr, Datiles MD, Cobo LM, and Kupfer C: Aldose reductate and complications of diabetes. Ann Intern Med (in press).

Katz ML, Robison WG Jr, and Dratz EA: Potential role of antoxidation in age changes of the retina and retinal pigment epithelium of the eye. In Free Radicals in Molecular Biology. Armstrong D, Sohal R, and Cutler R, (editors), New York, Raven Press (in press).

Katz ML, Robison WG Jr: Antioxidant deficiencies as models for studying the role of autoxidation in senescence. In Free Radicals, Aging and Degenerative Diseases. Johnson JE (editor). New York, Alan R. Liss (in press).

Robison WG Jr, Kador PF, Akagi Y, and Kinoshita J: Basement membrane thickening in ocular vessels of galactosemic rats prevented with aldose reductase inhibitors. Invest Ophthalmol Vis Sci 25(Suppl):66, 1984.

Katz ML, and Robison WG Jr: Age-related and vitamin E-related lipofuscin in ocular and other tissues. Invest Ophthalmol Vis Sci 25(Suppl):208, 1984.

Katz ML and Robison WG Jr: Age-related alterations in retinal pigment epithelium basal plasma membrane morphology. AGE (in press).

Nagata M, Katz ML, and Robison WG Jr: Retinal capillary basal lamina thickening during senescence. AGE (in press).

Kador PF, Robison WG Jr, and Kinoshita JH: Pharmacology of aldose reductase inhibitors. Ann Rev Pharmacol Toxicol (in press).

Katz ML, and Robison WG Jr: Senescence and the retinal pigment epithelium: Alterations in basal plasma membrane morphology. Mech Age Dev (in press).

Massof RW, Sykes SM, Rapp LM, Robison WG Jr, Zwick H., and Hochheimer, B. Optical radiation damage to the ocular photoreceptors. In Long-term Visual Health and Optical Radiation. M Waxler, VM Hitchens (editors). Boca Raton, Florida, CRC Press, Inc. 1985 (in press).

Robison WG Jr, Kador PF, and Kinoshita JH. Sorbinil prevention of early retinal microangiopathy. Diabetic Med (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00193-01 LOP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Hereditary Eye Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	George Inana	M.D., Ph.D.	Medical Officer	LOP, NEI
Others:	Lorenzo Merola	M.S.	Chemist	LOP, NEI
	Takashi Shiono	M.D.	Visiting Associate	LVR, NEI
	Mary Stephenson	B.A.	Student	LOP, NEI
	Paul Matsumura		Summer Student	LOP, NEI

COOPERATING UNITS *(if any)*

(Toshihiro Ohura, Eiki Kominami, Nobuhiko Katunuma)
School of Medicine, Tokushima University, Tokushima, Japan

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ornithine Aminotransferase Deficiency in Gyrate Atrophy: Our goal is to isolate a molecular probe for the human ornithine aminotransferase (OAT) in the form of a cDNA molecule and to use it to investigate the nature of the OAT gene defect(s) present in gyrate atrophy patients. We have employed the expression cloning vector system, λgt11 for cDNA cloning; clones can be isolated from a λgt11 cDNA library by the Western screening method using a specific antibody directed against the protein product of the gene desired. Messenger RNAs (mRNAs) were prepared from human liver, retina, and retinoblastoma cells. λgt11 cDNA libraries were made with all three mRNA populations, and the libraries were screened by the Western method using the prepared anti-OAT antibodies. A putative OAT cDNA clone was isolated from the retinoblastoma cDNA library which gives positive reactions with anti-OAT antibodies from two different sources.

Hereditary Retinoblastoma: We have begun to investigate the molecular basis of malignant transformation in hereditary retinoblastoma using cell culture and molecular genetic techniques. In order to test for the presence of "activated" oncogene(s) in retinoblastoma, we have transfected the chromosomal DNA of retinoblastoma cells (Y79) on various types of cells in culture. The transfections on NIH 3T3, CV-1, and newborn mouse retinal cells have not yielded any transformed cell phenotype. In view of the published data indicating that induction of hereditary retinoblastoma may involve a loss or inactivation of a gene on chromosome 13, we are also investigating the possibility that the genomic DNA from normal human retina, when transfected on retinoblastoma cells, may be able to change the phenotype of retinoblastoma to that of a more "normal" cell. To determine if retinoblastoma has a dominant or recessive malignant phenotype, retinoblastoma cells are being fused with normal cells, and the growth characteristics of the hybrid cells are being studied.

Project Description:

Additional Personnel Engaged on Project: None

Objectives:

OAT Deficiency in Gyrate Atrophy: The objectives of this project are to isolate a molecular probe for the human ornithine aminotransferase (OAT) and to use it to study the nature of the OAT gene defect(s) present in gyrate atrophy patients.

Hereditary Retinoblastoma: The objectives of this project are to isolate and study the gene(s) involved in the malignant transformation of retinoblastoma and, therefore, to understand the molecular mechanisms of this genetic malignant eye disease.

Methods Employed: All available methods of protein and nucleic acid analysis are used. These include polyacrylamide and agarose gel electrophoresis, immunoprecipitation, Western blotting and screening, column chromatography, isoelectric focusing, amino acid sequencing, DNA and RNA isolation, molecular cloning, DNA and RNA hybridizations, in vitro translation, cell culture techniques, DNA mediated gene transfer, cell fusion and DNA sequencing. The proteins and nucleic acids are obtained from human tissue and antisera from animals.

Major Findings:

OAT Deficiency in Gyrate Atrophy:

1. The OAT mRNA appears to be present in very low abundance in human liver and retina mRNA populations as measured by in vitro translation and immunoprecipitation.

2. The retinoblastoma cells (Y79) contain relatively high level of OAT mRNAs as compared to human liver and retina.

3. A putative OAT cDNA clone has been isolated from an expression library; the protein product encoded by the clone is reactive with anti-OAT antibodies from two different sources.

Hereditary Retinoblastoma:

1. Chromosomal DNA from retinoblastoma cells was not able to transform NIH 3T3, CV-1, or newborn mouse retina cells in culture.

2. Preliminary data suggest that fusion of retinoblastoma and normal cells results in a hybrid cell which shows a normal, non-malignant growth characteristic.

Significance to Biomedical Research and the Program of the Institute:

OAT Deficiency in Gyrate Atrophy:

Gyrate atrophy is significant among the hereditary diseases of the retina in that the specific biochemical defect, the OAT deficiency, is known. Thus, this disease offers a special opportunity for one to investigate the molecular basis of a hereditary retinal disease at the gene level by employing the latest molecular genetic techniques. The isolation and study of the normal and abnormal OAT genes will not only help us to understand the molecular basis of gyrate atrophy but may also provide useful information for genetic counseling and possible gene therapy of this blinding disease.

Hereditary Retinoblastoma:

Retinoblastoma is the most significant ocular tumor of children. It is a unique malignant disease in that approximately 40% of the cases manifest a hereditary pattern, and a specific chromosomal abnormality has been implicated in the disease. Thus, retinoblastoma is a prototype of genetic cancers and offers a great opportunity to study the molecular genetic basis of a malignant disease. The elucidation of the molecular genetic basis of this disease will most likely help in early diagnosis and genetic counseling of the patients, and may pave the way for a possible gene therapy technology in the future.

Proposed Course: The following studies are in progress or proposed for FY 1985.

OAT Deficiency in Gyrate Atrophy:

1. Identification of the isolated cDNA clone by hybrid-selected translation and DNA sequencing.
2. Partial amino acid sequencing of the purified OAT.
3. Determination of OAT RNA and DNA profiles in normal subjects and gyrate atrophy patients using the isolated molecular probe.
4. Isolation and analysis of OAT genes from normal and affected subjects.

Hereditary Retinoblastoma:

1. Development of a selection system for a "normal revertant" of the retinoblastoma cells in combination with further assay of normal human retina genes on retinoblastoma cells.
2. Use of DNAs cloned in mammalian expression vectors for transfection on retinoblastoma and non-retinoblastoma cells.
3. Analysis of hybrid cells formed by fusion of retinoblastoma and non-transformed cells.
4. Analysis of human retina cDNA clones which hybridize to normal human retina mRNA but not to retinoblastoma mRNA.

NEI Research Program: Retinal and Choroidal Diseases- -Developmental and Hereditary Disorders (Tumors)

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00194-01 LOP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Crystallin Abnormalities in the Philly Mouse Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	George Inana	M.D., Ph.D.	Medical Officer	LOP, NEI
Others:	Deborah Carper	B.S.	Biologist	LVR, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

We have been conducting a molecular genetic investigation of crystallin abnormalities present in the Philly mouse lens. The Philly mouse develops dominantly inherited cataracts beginning approximately 30 days after birth, at which time a generalized decrease of all crystallins is also observed. A specific deficiency of a 27,000 dalton (27K) β-crystallin polypeptide and its functional mRNA was demonstrated in the pre-cataractous 10-day-old Philly mouse lens. Total RNAs and polyadenylated mRNAs were isolated from 20 to 30-day-old normal and Philly mouse lenses and analyzed by in vitro translation and Northern blot hybridization. There was no difference in the translational efficiency of the normal and Philly total RNA's, but a marked decrease in the translational efficiency of the Philly mRNAs compared to the normal mRNAs was noted. A specific, severe decrease in the translation of the 27K β-crystallin polypeptide was observed. The hybridization of murine α, β, and γ-crystallin cDNA probes to the Northern blots of total RNAs and mRNAs from the normal and Philly lens demonstrated no difference in the levels of these crystallin RNA sequences in the total RNA populations, but a significant decrease in all of these crystallin mRNAs was present in the Philly mRNAs compared to the normal mRNAs. A marked deficiency in the level of the 23,000 dalton (23K) β-crystallin mRNA was observed. The hybridization of other murine β-crystallin cDNA probes to the mRNA Northern blots indicated that the 23K β-crystallin mRNA was most severely deficient in the Philly lens. A comparison of the in vitro translation products of the normal and Philly mRNAs with the hybrid selected translation products of the 23K β-crystallin cDNA suggested that the 23K β-crystallin, for which the cDNA and the gene have been isolated and studied, may be identical to the 27K β-crystallin polypeptide which is severely deficient in the Philly lens.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The objectives of this project are to investigate the molecular genetic basis of the crystallin abnormalities, especially that of the 23/27 K β-crystallin, present in the Philly mouse lens and to study their relationship to the cataractogenesis.

Methods Employed: All available methods of protein and nucleic acid analysis are used. These include polyacrylamide and agarose gel electrophoresis, immunoprecipitation, Western blotting and screening, column chromatography, isoelectric focusing, amino acid sequencing, DNA and RNA isolation, molecular cloning, DNA and RNA hybridizations, in vitro translation, and DNA sequencing. The proteins and nucleic acids are obtained from animals.

Major Findings:

1. Translational efficiencies of the total RNAs from the 20 to 30-day-old normal and Philly lens are similar.

2. Translational efficiency of the polyadenylated mRNAs from the 20 to 30-day-old Philly lens is significantly lower than that of the normal lens. A specific, severe deficiency of the 27K β-crystallin translation product is present.

3. The α, β-, and γ-crystallin RNA levels are similar in the total RNA populations of the 20-30 day old normal and Philly mouse lens.

4. The α-, γ- and especially the β-crystallin mRNA levels are significantly decreased in the mRNA population of the 20-30 day old Philly lens compared to the normal.

5. The 23K β- crystallin mRNA level is most severely deficient in the 20 to 30 day old Philly lens among the different β-crystallin mRNA levels examined.

6. The 23K β-crystallin, for which the cDNA and the gene have been isolated and studied, may be identical to the 27K β-crystallin, which has been shown to be deficient in the Philly mouse lens.

Significance to Biomedical Research and the Program of the Institute:
The Philly mouse, which develops hereditary cataracts, is a valuable animal model to investigate the molecular basis of cataractogenesis. The discovery of a specific deficiency in one of the β-crystallin polypeptides in the Philly lens pointed out the possibility that this may be a primary gene defect which could be causally related to the development of cataract. Since the crystallins are the main soluble proteins of the lens and are important for the maintenance of lens transparency, it seems logical to speculate that an abnormality in the crystallins would lead to cataract formation. The elucidation of the molecular genetic basis of such a specific crystallin defect would serve to answer this question and may contribute to the understanding of the molecular basis of a disease process in the lens.

Proposed Course:

1. Further analysis of total RNAs and mRNAs from the normal and Philly mouse lens of different ages with respect to their translational efficiencies and content of different crystallin mRNAs.

2. Identification and isolation of a molecular probe for the 27K β-crystallin which is deficient in the Philly lens. In this regard, the 23K β-crystallin cDNA will be analyzed further.

3. Investigation of the molecular genetic basis of the 27K β-crystallin defect in the Philly lens including the isolation and study of defective gene(s).

NEI Research Program: Cataract--Nongenetic Congenital and Genetic Cataract and Dislocated Lenses (Nongenetic Congenital and Genetic Cataract)

Publications:

Carper D, Shinohara T, Piatigorsky J, and Kinoshita JH: Deficiency of functional messenger RNA for a developmentally regulated β-crystallin polypeptide in a hereditary cataract. Science 217:462, 1982.

Inana G, Shinohara T, Maizel JV Jr, and Piatigorsky J: Evolution and diversity of crystallins. J Biol Chem 257:9064, 1982.

Inana G, Piatigorsky J, Norman B, Slingsby C, and Blundell T: Gene and protein structure of a β-crystallin polypeptide in murine lens: relationship of exons and structural motifs. Nature 302:310, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00192-01 LOP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stromal Damage Following Removal of the Epithelium in the Rat Cornea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kiyoo Nakayasu	M.D.	Visiting Associate	LOP, NEI
Others:	Toichiro Kuwabara	M.D.	Chief, Laboratory of Ophthalmic Pathology	LOP, NEI
	Mary A. Crawford	B.A	Biological Lab Technician	LOP, NEI
	Timothy Schoen	B.S.	Biological Aide	LOP, NEI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Ophthalmic Pathology

SECTION

Section on Experimental Pathology

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Following removal of the epithelium using a newly developed gentle technique, the stroma was examined histologically. Removal of the epithelium itself caused degeneration of keratocytes in the anterior zone. Repair of the acellular stroma began in the posterior stroma about 24 hours after the operation. The cell membranes of the original basal epithelium, which remained intact for at least 12 hours, prevented leucocytes and water from getting into the stroma.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The objective of this project is to clarify cellular interaction between the epithelium and stroma cells of the cornea by studying cellular changes in the keratocyte following removal of the epithelium.

Methods Employed: The epithelium of SD strain albino rats was removed by gentle contact with a dried glass strip coated with 10% gelatin. The corneas were histologically examined at various time intervals using light and electron microscopy. Autoradiographic examination was also performed by tagging stroma cells with ^{3}H-thymidine which was injected into the anterior chamber at varying stages of the experiment.

Major Findings:

1. The present technique did not cause any trauma in the stroma. However, keratocytes situated beneath the denuded area degenerated soon after the operation and the anterior stroma become acellular approximately 12 hours later.

2. The basal cell membrane of the original epithelium remained intact for at least 12 hours and prevented wandering cells and water from getting into the stroma. Wandering cell infiltration occurred only for a short time when recovery by the sliding epithelium was near completion.

3. Repopulation of the acellular zone began in the posterior stroma about 24 hours after the operation. Keratocytes in this area showed active mitosis and marked incorporation with ^{3}H-thymidine.

Significance to Biomedical Research and the Program of the Institute: Although epithelial removal is a common clinical procedure, systematic studies on cytologic effects of this insult to the stroma are relatively sparse. This study has demonstrated a fundamental cellular alteration following epithelial removal and a healing mechanism in the corneal stroma.

Proposed Course: Further studies will be carried out to obtain more information on specific cellular reactions and on maintaining mechanism of the extracellular matrix after removal of the epithelium, especially by repeated operation.

NEI Research Program: Corneal Disease-Corneal Transplantation and Wound Healing

Publications:

Nakayasu K, Miller GE, and Kuwabara T: Corneal stroma damage following removal of the epithelium in rat. Invest Ophthalmol Vis Sci 25 (Suppl):327 , 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00078-07 LOP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histopathology of Human Dystrophies and Degeneration

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Merlyn M. Rodrigues,	M.D.	Chief, Section on Ophthalmic Pathology	LOP, NEI
Others:	Joseph Hackett	B.S.	Biologist	LOP, NEI
	Reginald Gaskins		Histologist	LOP, NEI

COOPERATING UNITS *(if any)*
Department of Ophthalmology, University of Iowa, Iowa City (J. Krachmer).

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Ophthalmic Pathology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human corneal dystrophies and degenerations which have been clinically documented are studied as keratoplasty specimens with histochemical stains, scanning and transmission electron microscopy, and immunologic techniques in an attempt to elucidate pathogenetic mechanisms. This approach has provided insight into cell-to-cell relationships in the normal and diseased states. In patients with primary and recurrent macular corneal dystrophy, intercellular and extracellular accumulation of fibrillogranular material was observed in the corneal stroma, Descemet's membrane, and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase have been investigated with immunofluorescent, electrophoretic, and chromatographic methods. The lectin binding patterns were compared in corneas from patients with macular dystrophy and controls.

The characterization of amyloid in lattice corneal dystrophy and corneal amyloid degeneration was performed using immunohistochemical stains and biochemical analyses. Keratoplasty specimens from granular corneal dystrophy and controls were examined by combinations of immunohistological stains, transmission electron microscopy, and SDS gel electrophoresis.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The study attempts to combine detailed clinical and genetic studies of patients with human and corneal diseases, particularly corneal dystrophies, to obtain further insight into the mechanisms of corneal opacification.

Methods Employed: Corneal specimens from transplant patients are divided into portions and used separately for light, scanning, and transmission electron microscopy. These data provide insight into the morphological appearance of the cells and extracellular materials of the corneal layers. Other portions of the surgical specimens are placed into tissue and cell culture to allow examination of the morphology and biosynthetic activities of the cells of the three corneal layers. Indirect immunofluorescence has shown the range of collagen types present in normal and abnormal tissue. Column chromatography and electrophoresis provide information about the collagen, glycoconjugates, phospholipid, and biosynthetic patterns of abnormal tissue. Two-dimensional gel electrophoresis and the Western blot technique were used for the analysis of amyloid.

Major Findings:

1. Corneal Dystrophies: Keratoplasty specimens from eight patients with granular corneal dystrophy (GCD) and age-matched controls were examined by combinations of immunohistological stains, transmission electron microscopy (TEM), and SDS gel electrophoresis. Fresh frozen sections from GCD corneas stained positively with antibodies to microfibrillar protein by immunofluorescence. Routine TEM revealed that the granules had central electron-dense areas partially surrounded by 9-10 nm tubular microfibrils. Material eluted from GCD corneas showed denser peptide bands at 65K and 110K than that from corneas. Stains were negative for elastin, amyloid, neutral lipids, cholesterol, and glycosaminoglycans. Luxol fast blue stain was strongly positive in the granules in all cases examined. Immunofluorescent stains were negative with antibodies to fibronectin, laminin, collagens I-V, basement membrane proteoglycan, tropoelastin, and keratin. In two GCD corneas, an increased lipid content was found in every phospholipid class although cholesterol content was unchanged. An unusual form of GCD with superficial confluent opacities was also observed.

2. Corneal Degenerations: (1) Keratoconus specimens had the same range of collagen types as normal cornea, with type I collagen predominating. Type III collagen was detected only in scarred areas. Radioactive-labeling experiments on cultured cells from these corneas have demonstrated an elevated production of collagenase compared with the normal. (2) Pellucid corneal degeneration showed thinned cornea inferiorly with no evidence of vascularization. Light and electron microscopy of a corneal button from each patient revealed irregularity of the epithelium in the peripheral thinned areas, with a normal Bowman's layer in one case and focal dehiscences in the other. Marked thinning of the corneal stroma accompanied by the presence of a small number of histiocytes was present peripherally in both bases. Descemet's membrane and endothelium were normal. Stromal collagen was normal in diameter and periodicity. In one case, CM-cellulose and SDS gel profile of the collagens synthesized by these stromacytes in vitro (^{3}H proline label) was similar to those of control corneas and keratoconus.

3. Lack of Evidence for AA Reactivity in Amyloid Deposits of Lattice Corneal Dystrophy and Amyloid Corneal Degeneration. Amyloid fibrils occurring in primary or myeloma (AL), secondary (AA), and certain neutropathic hereditary forms of systemic amyloidosis can be distinguished biochemically and/or immunohistologically as being composed of immunoglobulin light chain (L-chain), AA protein, or prealbumin (Pa). All types of systemic as well as several localized forms of amyloidosis contain amyloid P component (AP protein). Fixed tissue from eight cases of hereditary lattice dystrophy were studied by the immunoperoxidase method using two antisera to AA and AP, to serum prealbumin (Pa) isolated from a case of hereditary amyloidosis, and to L-chain determinents; additional cases were examined by indirect immunofluorescence of fresh material. Weak (1:10 dilution) staining with anti-AP was found, but no reactivity with other antisera was found. Congo red stain and dichroism was resistant to pretreatment of sections with potassium permanganate, a characteristic of non-AA amyloid. Two dimensional (2D) gels of solubilized proteins from frozen tissue of two cases of LCD resembled those obtained from normal human cornea (NHC). Western blots of two cases of corneal amyloid degeneration and NHC solubilized protein did not react with I^{125} labelled anti-AA or AP, utilizing purified AP protein and AA amyloid tissue as controls. We are unable to corroborate the presence of AA protein in LCD; although staining with antiserum to AP protein is demonstrable, its molecular configuration in stromal deposits remains to be defined.

4. C-reactive protein in lattice corneal dystrophy. Serum amyloid P component (SAP) and C-reactive protein (CRP) resemble each other in molecular structure and amino acid sequence, but appear to be antigenically distinct. In this study, corneas from normal controls, primary LCD and recurrent LCD were fixed in formalin with 1mM $CaCl_2$ and tested with antibodies to CRP, AP, and AA (nonimmunoamyloid) using the immunoperoxidase technique. The stroma of LCD and normal corneas did not stain with antibodies to AP, AA, or CRP. However, we now report that antibodies to CRP show immunospecific binding to the corneal epithelium in primary and recurrent LCD.

Significance to Biomedical Research and the Program of the Institute: The mechanisms of opacification and destruction of the cornea in a variety of human diseases must be understood for the improved diagnosis and classification of these entities. This may also lead to a more rational basis for the appropriate treatment of these visually disabling processes. A thorough knowledge of the genetic components of these disorders, if any, will aid in more effective and complete genetic counselling.

Proposed Course: Patient material will be entered into this combined study as it becomes available. Emphasis will be placed on elucidating pathogenic mechanisms in hereditary posterior polymorphous dystrophy, keratoconus, and lattice and granular dystrophies. The use of immunohistological techniques will be expanded to a wider variety of specimens.

NEI Research Program: Corneal Diseases-Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Diseases (Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies).

Publications:

Rodrigues MM, Gaster R, and Pratt M: Unusual superficial confluent form of granular corneal dystrophy. Ophthalmology 90:1507, 1983.

Rodrigues M, and Robey P. C-reactive protein in human lattice corneal dystrophy. Curr Eye Res 2:721, 1983.

Gorevic PD, Rodrigues, MM, Krachmer JH, Green C, Fujihara S, and Glenner G: Lack of evidence for AA reactivity in amyloid deposits of lattice corneal dystrophy and amyloid corneal degeneration. Am J Ophthalmol 98:216, 1984.

Purcell JM, Rodrigues M, Chisti MI, Riner RN, and Dooley MM: Lattice corneal dystrophy associated with familial systemic amyloidosis (Meretoja's syndrome). Ophthalmology 90:1512, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00096-06 LOP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinicopathologic Studies of Human Ocular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Ophthalmic Pathology	LOP, NEI
Others:	Joseph Hackett	B.S.	Biologist	LOP, NEI
	Reginald Gaskins		Histologist	LOP, NEI
	Nicole Newman		Histologist	LOP, NEI
	Gunter Thomas		Biologist	LOP, NEI

COOPERATING UNITS *(if any)*
Wills Eye Hospital, Philadelphia (G. Spaeth).

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Ophthalmic Pathology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with localized ocular diseases or with ocular manifestations of systemic disease are examined clinically, and photographic documentation is made of significant findings. Biopsy specimens or autopsy eyes from these patients are examined by scanning and transmission electron microscopy and histochemical stains. Studies are performed on patients with ocular manifestations of systemic diseases.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Studies of the morphology of tissue specimens as well as cells cultured from normal and abnormal ocular tissues are essential for further insights into possible pathogenetic mechanisms of disease. The utilization of immunohistochemical methods and histochemical stains is also helpful in the diagnosis of certain conditions.

Methods Employed: Specimens are obtained from patients at the National Eye Institute Clinic as well as other ophthalmic centers in the United States. In most instances, specimens are processed by appropriate techniques for histology, histochemistry, and electron microscopy. Selected specimens are frozen for special immunological studies. In other cases, routine histopathology is performed.

Major Findings:

1. Ocular manifestations of systemic diseases

a. Acquired Immunodeficiency Syndrome (AIDS)

Forty patients with acquired immunodeficiency syndrome (AIDS) were examined for ocular abnormalities. Twenty of these patients died, and the eyes were obtained for culture and histopathologic examination. These patients have multiple opportunistic infections and neoplasms as the result of a severe depression of cellular immunity. Fifty percent of all patients with AIDS and 75% of the autopsy group have ocular signs attributable to AIDS. Ocular findings fell into four major categories: cytomegalovirus retinitis (10 patients), retinal cotton wool spots (11 patients), conjunctival Kaposi's sarcoma (2 patients), and neuro-ophthalmic motility abnormalities (3 patients). Cytomegalovirus retinitis was a significant cause of visual loss. Seven of 40 autopsy eyes had hand-motion or worse vision prior to the patients' death because of CMV and progressed to involve the entire retina in three to six months resulting in a gliotic retinal membrane.

2. Primary open-angle glaucoma

Surgical trabeculectomy was performed on 10 eyes of 10 patients following failure of argon laser trabeculoplasty. All specimens were examined by scanning electron microscopy to determine the anatomic location of laser damage. Correlation with clinical data revealed accurate placement of laser burns in 11 out of 13 treatment sessions. The degree of laser injury varied with energy delivered and pigmentation of the posterior trabecular meshwork.

3. a. Sympathetic Ophthalmia

An immunohistological study using monoclonal antibodies directed at specific membrane antigens of various inflammatory cells was carried out to evaluate the identity and topographical localization of the immunocompetent cells in an enucleated eye from a six-year-old black patient with a three-

month history of sympathetic ophthalmia. Correlative light transmission electron microscopic examination of serial sections was also performed. The data demonstrated that the predominant cells within the choroidal infiltrate were T lymphocytes (leu 1^+). T-cell subset analysis disclosed that most of these cells harbored specific antigenic determinants of the helper phenotype (Leu $3a^+$). A smaller proportion of the T cells demonstrated the specific determinants of the suppressor subtype (Leu $2a^+$). The helper/ suppressor ratio varied slightly and ranged in most areas of the choroid between 3:1 to 4:1. Additionally, approximately 15% of the infiltrating lymphocytes harbored the Leu 14^+ determinant specific for B cells. The latter were located in the outer choroid adjacent to the sclera. Very few natural killer (NK) cells (Leu 7^+) were identified throughout the choroid. The granulomatous foci in the choroid were composed mainly of epithelioid cells and histiocytes expressing the $OKMI^+$ and $M221^+$ antigenic determinants on their membranes and demonstrating a high cytoplasmic nonspecific esterase activity ($ANAE^+$). Within the Dalen-Fuchs nodules, similar to the choroidal nodules, there was a predominance of histiocytes and epithelial cells ($OKMI^+$, $M221^+$ and $ANAE^+$), a few T-helper cells (Leu 1^+, Leu $3a^+$), and some $OKMI^-$, $M22^-$ cells whose origin could not be determined. These findings were corroborated by electron microscopic observations of serial sections. Careful light and electron microscopic studies disclosed breaks in Bruch's membrane underlying some of Dalen-Fuchs nodules. In our opinion these observations may be interpreted as the demonstration that Dalen-Fuchs nodules and the choroidal granulomatous foci could be formed by identical cells of similar function and origin.

b. Pathology of idiopathic midline destructive disease (IMDD) in the eyelid

Subcutaneous eyelid and brow nodules were noted in a 25-year-old man who at 14 years had developed the central facial and upper airway necrosis characteristic of idiopathic midline destructive disease (IMDD). One large nodule was located near the lateral canthus rather than a midline position. Insofar as we know, this report would constitute the first evidence of IMDD in the eyelids and brow in the absence of orbital disease.

c. Histopathology of chorioretinal scars in chronic granulomatous disease

A 17-year-old boy with multiple recurrent systemic bacterial and fungal infections had bilateral chorioretinal scars inferiorly. Light microscopy disclosed almost total atrophy of choroid and retina in the larger opaque portions and irregular proliferation of retinal pigment epithelium at the scar edges. Special stains, for ocular organisms and cultures from the fresh autopsy eyes were negative.

Significance to Biomedical Research and the Program of the Institute: These studies are directly concerned with mechanisms involved in primary and secondary glaucoma; corneal, conjunctival and retinal diseases, and ocular manifestations of systemic diseases.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Glaucoma-Primary open-angle Glaucoma (Aqueous Humor Dynamics: Outflow)

Publications:

Rodrigues MM, Palestine AG, Macher AM, and Fauci AS: Histopathology of ocular changes in chronic granulomatous disease. Am J Ophthalmol 96:810, 1983.

Palestine AG, Rodrigues MM, Macher AM, Chan CC, Lane HC, Fauci AS, Masur H, Longo D, Reichert CM, Steis R, Rook AH, and Nussenblatt RB: Ophthalmic involvement in acquired immunodeficiency syndrome. Ophthalmology 91:1092, 1984.

Chu FC, Rodrigues MM, Cogan DG, Barranger JA, and Stowens DW: The pathology of idiopathic midline destructive disease (IMDD) in the eyelid. Ophthalmology 90:1385, 1983.

Chan C, BenEzra D, Rodrigues MM, Palestine AG, Hsu S-M, and Nussenblatt RB: Immunohistochemistry and electron microscopy of choroidal infiltrates and Dalen-Fuchs nodules in sympathetic ophthalmia. Ophthalmology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00114-04 LOP

PERIOD COVERED
September 30, 1983 to October 1, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histopathologic Studies of Animal Models of Human Ocular Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Merlyn M. Rodrigues	M.D.	Chief, Section on Ophthalmic Pathology	LOP, NEI
Others:	James Jester	Ph.D.	Expert	LOP, NEI
	Reginald Gaskins		Histologist	LOP, NEI
	Joseph Hackett	B.S.	Biologist	LOP, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Ophthalmic Pathology

SECTION
Section on Ophthalmic Pathology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
- [X] (a) Human subjects
- [ ] (b) Human tissues
- [ ] (c) Neither
- [ ] (a1) Minors
- [ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the rabbit model, different aspects of corneal wound healing were evaluated. Immunofluorescent studies of experimental keratophakia showed that implanted lyophilized lenticules did not stimulate fibronectin synthesis or keratocyte repopulation by adjacent keratocytes. Alterations in keratin and actin proteins in regenerating corneal epithelium were also evaluated.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Light and electron microscopic examinations of eyes and tissues in these models provide essential information related to the possible pathogenesis of certain ocular developmental defects. Animal models are also useful for testing the efficacy of surgical techniques.

Methods Employed:

1. Histopathologic and Ultrastructural Studies of Cryorefractive Surgery in Humans and Animals: An albino rabbit model of keratophakia was also studied by light and electron microscopy and by immunofluorescent microscopy to better define the course of wound healing following this type of surgery. Localization of fibrin and fibronectin during healing after keratophakia in rabbit eyes was evaluated from 4 hours to 3 months after surgery. The results were compared to corneal wound healing following freeze injury to test the hypothesis that keratophakia lenticules exhibit a primary defect in wound healing that leads to a failure of host keratocytes to migrate into and repopulate the implanted tissue.

2. Expression of Keratin Proteins During Epithelial Wound Healing: Expression of keratin proteins by corneal epithelial cells were evaluated in albino rabbits following full-thickness trephination wounds and transcorneal freeze injury. Corneas were evaluated by light and electron microscopy, and epithelial keratin expression was evaluated by immunofluorescent techniques using mouse monoclonal antibodies to human epidermal keratins. Wounds were evaluated at various intervals from 4 hours to 2 months to test the hypothesis that regenerating corneal epithelial cells exhibit changes in the expression of keratin proteins during re-epithelialization and epithelial re-attachment to the underlying stroma.

3. Actin Filament Localization in Normal and Migrating Rabbit: Corneal Epithelium: The molecular probe NBD phallacidin (7-nitrobenz-2-oxa-1, 3-diazolyl-phallacidin), which reacts specifically with filamentous actin (f-actin), was used to study the distribution of polymerized actin oligmers in normal and migrating rabbit corneal epithelial cells.

Major Findings:

1. Model of Keratophakia: Keratocytes failed to regenerate in the rabbit model of keratophakia even when tissue was implanted into actively regenerating corneas. This suggests that keratophakia lenticules failed to attract normal keratocytes. Fibrin and fibronectin were not found in appreciable amounts in either freeze-injured corneal stroma or in the implanted lenticles. This suggests that these factors do not play a role in keratocyte regeneration in vivo.

2. Expression of Keratin Protein in Corneal Wound Healing: There was a marked change in the expression of keratin proteins in regenerating corneal epithelial cells, as identified by AE1 monoclonal keratin antibody which recognizes a class of keratin proteins present normally only in the superficial corneal epithelium. During corneal wound healing, all epithelial cells overlying

the wound reacted positively with AE1 antibody, suggesting that these cells synthesize a new keratin protein which may be linked to the wound healing process. Return to a normal expression of keratin proteins was correlated with reattachment of the epithelium to the underlying stroma which required weeks in the case of freeze injury and months in the case of trephination injury.

3. Actin Filament Localization in Normal and Migrating Rabbit Corneal Epithelium. In the normal cornea, the majority of the NBD phallacidin fluorescence was localized to the cortical or sub-plasma membrane area of the superficial and wing epithelial cells. Following full thickness corneal trephination injury, migrating corneal epithelial cells exhibited a marked increase in the cortical NBD phallacidin fluorescence. Transmission electron microscopy, using fixation techniques which decrease the disruption of filamentous actin, revealed bundles of fine filaments (6nm underlying the plasma membrane of migrating epithelial cells, thus supporting the fluorescent results. These findings suggest that corneal re-epithelialization is characterized by a marked increase in the amount of filamentous actin within the migrating epithelial cells. We conclude that NBD phallacidin may be of value in analyzing changes in actin polymerization during wound healing.

Significance to Biomedical Research and the Program of the Institute: The change in keratin expression observed in corneal re-epithelization may provide a marked for epithelial regeneration and a marker for poor epithelial reattachment to the cornea. In cryorefractive surgery, epithelial reattachment and reduplication of basal lamina may be a serious complication. The data suggest that fibronectin and fibrin do not play a significant role in keratocyte regeneration and are not responsible for the failure of keratocytes to repopulate keratoplakia lenticules.

Proposed Course: Studies of animal models will continue in the next fiscal year.

NEI Research Program: Corneal Diseases-Corneal Transplantation and Wound Healing (Inflammation and Repair).

Publications:

Jester JV, and Rodrigues MM. Actin filament localization in normal and migrating rabbit corneal epithelium. Curr Eye Res 3:955, 1984.

Jester JV, Rodrigues MM, Villasenor RA, and Schanzlin DJ. Keratophakia and keratomileusis: Histopathologic, structural and experimental studies. Ophthalmology 91:793, 1984.

LABORATORY OF SENSORIMOTOR RESEARCH

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE CHIEF, LABORATORY OF SENSORIMOTOR RESEARCH

Robert H. Wurtz, Ph.D.

In this sixth Annual Report of the Laboratory of Sensorimotor Research, I would like to emphasize work in the laboratory related to saccadic eye movements. These rapid eye movements displace the eye quickly from one part of the visual field to another, and are essential to normal vision, particularly in such tasks as reading. While concentration on saccades in this report neglects other work described in the individual reports, it allows emphasis of salient recent discoveries made in the laboratory. The diverse disciplines of single-unit neurophysiology, psychophysics and biomedical engineering have been applied in our laboratory to study the sensory processes underlying saccade initiation, the motor systems responsible for generating saccades, and the central, adaptive mechanisms that compensate for deficits in saccadic performance caused by aging, injury, and disease.

Since the discovery here over a decade ago that cells in the superior colliculus in the brainstem of the monkey discharge before saccadic eye movements, the colliculus has become recognized as central to the initiation of saccades. Two other structures provide the anatomically most prominent input to the superior colliculus: the frontal eye fields of the cerebral cortex, and the substantia nigra of the basal ganglia, and both of these brain structures have been studied within the laboratory.

Dr. Goldberg's group has analyzed how the frontal eye fields of monkeys control visually guided eye movements, providing one of the most coherent and elegant descriptions of sensorimotor processing in the cerebral cortex. They have previously described three modes of neural activity preceding purposive saccadic eye movements: visual, movement, and anticipatory, and they have shown how activity before a saccade begins with an anticipatory discharge if the animal can predict the direction of the saccade it will have to make, includes activity dedicated to visual analysis and the selection of targets for eye movement, and concludes with activity which could serve as targeting and triggering signals for lower oculomotor centers to make the saccade. The hallmark of this movement activity is that it occurs only before saccades that are purposive: it does not occur before eye movements that occur spontaneously in the dark. Lesions of the frontal eye fields, previously thought not to affect oculomotor behavior, have now been shown to have a devastating effect not on simple visually guided eye movements, but on the monkey's ability to learn more difficult oculomotor tasks. These experiments on frontal eye fields have led Dr. Goldberg to the hypothesis that the programming of saccadic eye movements made under complex conditions requires the participation of the frontal eye fields although the programming of a single visually guided saccade can be done by the superior colliculus alone.

Recent experiments by Dr. Wurtz and his group have for the first time established the role of the diencephalic structure, the basal ganglia, in the

initiation of saccadic eye movements. The output of this structure, the substantia nigra pars reticulata, projects to the superior colliculus as do the frontal eye fields. Like cells in the frontal eye fields, these cells in the substantia nigra discharge under highly specific conditions: in relation to saccades made to visual targets and to those made to remembered targets, but not in relation to eye movements made spontaneously in the dark. The response of these cells is a pause in a high background discharge rate, the action of these cells on the superior colliculus is inhibitory, and the signal conveyed is therefore a brief release of inhibition with each saccade. The inhibition is likely to be produced by the neurotransmitter GABA and this offered an opportunity so far unique in the oculomotor control system: analysis of function by selective alteration of transmitter action. They found that injection of GABA into the superior colliculus produced a paucity of saccades by increasing inhibition, particularly with saccades to remembered targets, and a flurry of irrepressible saccades when the inhibition is blocked. These experiments in the monkey suggest a mode of action of the basal ganglion in relation to eye movements: a tonic inhibition, released at the time of saccades, particularly those made in the absence of direct sensory control. This interpretation of basal ganglia function found in monkeys has been used to test human patients with a disease of the basal ganglia, Parkinson's disease. As is the case for the cells in the monkey substantia nigra, patients with Parkinson's disease show a greater deficit with eye movements made to remembered targets than to visual targets. Thus, experiments originally designed to analyze single cells in the monkey reveal a deficit in oculomotor control in human patients.

In making saccadic eye movements, some selection of the visual target of the eye movement must be made; the eye can move in only one direction at a time. This selection process in its more general form is a type of selective visual attention, and Dr. Robinson and his collaborators have been able to investigate in the monkey an attentional phenomenon first reported in man. They have shown that in monkeys and in man, the reaction time to a visual target is improved if the subject is given a cue as to the future location of the target. Cues which invalidly indicate the target location slow reaction times. In their continuing analysis of the pulvinar nucleus of the monkey thalamus, they observed single neurons which show visual and behavioral properties which might be related to such selective visual attention. They therefore attempted to block or facilitate neural activity in this structure and then test the monkey's performance on the attention task. Since GABA is also a neurotransmitter in this part of the brain, they used GABA-agonists and antagonists. A GABA-antagonist produced difficulty in shifting attention to the contralateral visual field while a GABA-agonist produced a facilitation in the shift of attention to the contralateral field. These results are the first clear demonstration of a function for the pulvinar, a large structure in the visual system with no known function. Furthermore, they are the first demonstration of an alteration of visual spatial attention by a highly selective but reversible lesion in the monkey.

Dr. Miles and his collaborators have discovered an ocular following response to sudden movements of the visual scene that occurs after saccadic eye movements. These responses help to prevent any drift of the eyes following a saccade and thereby stabilize gaze and produce better visual acuity. They found that these ocular following responses in monkeys occur with surprisingly short latency--approximately 50 msec--and that they are transiently enhanced following saccadic eye movements. They have now shown that the enhancement is the result of the visual stimulation generated by the saccade sweeping the retina across the visual

scene. When a saccade is made in the absence of appropriate visual stimulation, as when a vertical saccade is made while viewing vertical stripes, no enhancement results. On the other hand, the enhancement can be evoked in the absence of a saccade simply by shifting the visual scene in a manner that mimics the visual events occurring during saccades. Furthermore, since there is no interocular transfer of the visual enhancement, the effect clearly occurs at early stages of visual processing. In net, these experiments demonstrate a new visual enhancement phenomenon in the monkey's oculomotor system that may play a significant role in the visual stabilization of the eyes following saccadic displacements.

A full understanding of the saccadic motor system requires insight into both the movement generator and the adaptive mechanisms that maintain its performance. Dr. Optican has been studying patients and monkeys to understand the neural organization of the saccadic motor system and the nature of its adaptive control. Two testable hypotheses, expressed as mathematical models, have recently been developed. One model concerns the mechanism underlying the generation of eye movements in congenital nystagmus, and shows how a defect in a single element of the brain stem ocular motor system can account for many of the clinical features of congenital nystagmus. The model also identifies just two parameters as being clinically useful (initial slow phase velocity and time constant), both of which can be measured with clinical eye movement monitors. The second model concerns the adaptive suppression of post-saccadic ocular drift. Experiments have revealed that the time constant of ocular drift elicited by prolonged exposure to retinal image slip depends linearly on the time constant of the adapting stimulus. This violates an earlier hypothesis that such drift is dependent only on the dynamics of the orbital elements (the ocular plant). Dr. Optican developed a new description of the final common path of the ocular motor system (brain stem network and ocular plant) that makes the saccadic innervation a mixture of phasic and tonic components in a pulse-slide-step combination. The model shows that suppression of post-saccadic ocular drift depends on adaptive control of three parameters: the gain of the step and both the gain and the time constant of the slide of innervation. These new models are sufficiently detailed that they can be used in the computer simulation of ocular motor behavior to test hypotheses about normal and abnormal saccadic function in monkeys and human patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00049-06 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebral Cortical Mechanisms for Eye Movements and Visual Attention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael E. Goldberg	M.D.	Chief, NMS	LSR, NEI
Others:	Mark Segraves	Ph.D.	Staff Fellow	LSR, NEI
	Deng Shu-yi	M.D., Ph.D.	Visiting Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Neuro-Ophthalmologic Mechanisms Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	1.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The frontal eye fields of the cerebral cortex contain neurons which discharge before saccadic eye movements. Previous work from this laboratory has shown that the region of the frontal eye fields with presaccadic cells projects to the superior colliculus in an orderly fashion. Electrical stimulation of the superior colliculus excites presaccadic neurons in the frontal eye fields antidromically. Electrical stimulation of superior colliculus regions participating in the genesis of saccades greater than 10^{o} results in saccades whose amplitude and direction are affected by the orbital position of the eye. Stimulation of the superior colliculus also results in head movements whose parameters are affected by gaze position. The saccades evoked by electrical stimulation of the superior colliculus from identical orbital positions can be affected by the position of the animal's head relative to the midline of the body.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Previous work in this laboratory established that the frontal eye fields of the rhesus monkey contain neurons which could participate in the control of purposive eye movements. Because the frontal eye fields do not project directly to the eye muscle motor nuclei, but rather to other oculomotor integrative areas, we focused on one of these projection areas, the superior colliculus, in order to analyze how cortical information is transformed by lower structures in the generation of oculomotor behavior. In order to study the relationship between the frontal eye fields and the superior we asked if neurons in the frontal eye fields could be driven antidromically from the superior colliculus. Because one of the most important findings establishing the role of the frontal eye fields in the generation of eye movements is the fact that electrical stimulation at the site of movement related frontal neurons evokes saccades predicted by the properties of the neurons, we examined the saccades evoked by electrical stimulation of the superior colliculus, especially to see if orbital position has an effect on either the eye movements evoked by electrical stimulation of the superior colliculus or the properties of single neurons.

Methods Employed: A digital computer was used for behavioral control, data acquisition, and on-line analysis of monkey behavior, eye movement, and neuronal discharge time patterns. In addition, software was developed for the on-line computer analysis of neuronal discharge morphology for the purpose of antidromic stimulation and collision studies on single neurons. Monkeys were trained to perform a series of visuomotor tasks, including visual fixation, saccadic eye movements to visual stimuli, eye movements to remembered points, and smooth pursuit eye movements. Microelectrodes were used to record the activity of single cells during these tasks, and microampere amounts of current passed through the recording microelectrode at the sites of the cells, in order to elicit saccades. Such eye movements were evoked while the monkey looked at central and eccentric targets. In certain experiments two microelectrodes were implanted simultaneously, one to record cellular activity in the frontal eye fields and the other for electrical stimulation and single neuron recording in the superior colliculus. Eye position and in some cases head position were simultaneously measured using the magnetic search coil technique, so that accurate quantitative measures of gaze position and velocity could be obtained. Records were stored on magnetic disk during the experiments and then transferred to magnetic tape for further analysis.

Major Findings: The functional importance of the projection from the frontal eye fields to the superior colliculus has been confirmed by the demonstration that axons of neurons which discharge before saccades can be electrically driven from the area of the superior colliculus from which similar saccades can be evoked.

Small saccades ($<10^{o}$) evoked from the superior colliculus are generally unaffected by where the eye was in the orbit when the stimulation occurred. At points from which larger saccades can be evoked the direction and amplitude of stimulation-evoked saccades become increasingly dependent upon initial orbital position. As initial orbital position moves in the direction opposite the direction of the saccade, the saccades grow longer. In addition, the direction of the saccade rotates; each site has an optimal axis. Saccades evoked from orbital

positions above the axis are rotated downward relative to the axis. Saccades evoked from orbital positions below the axis are rotated upwards.

When the superior colliculus is stimulated with the monkey's head free to move, a gaze shift including both an evoked eye movement and an evoked head movement occurs. The amplitudes of both the eye movement and the head movement are dependent not only on where the eye is in the orbit but also where the eye is relative to the midline of the body. Even saccades evoked from the same orbital position change their magnitudes according to gaze position. The receptive fields and movement fields of the cells recorded at the stimulation site do not share this dependence upon orbital position, but instead are consistent with the largest saccades evoked from the site. The demonstration that orbital and head position affect the saccades evoked by electrical stimulation of the superior colliculus implies that the cortical message is subject to modification by signals dealing with eye and head position, and that the oculomotor system has a hitherto unsuspected layer of complexity as it turns the location of the target for an eye movement into the movement to acquire that target.

Significance to Biomedical Research and the Program of the Institute: By understanding the mechanisms by which the cerebral cortex communicates with lower structures in the generation of visual and oculomotor behavior, we can begin to understand the basic mechanisms by which the brain integrates sensory information into motor programs. We can also begin to understand the mechanisms of the deficits in patients with lesions in visual-motor control areas, and use this understanding for improved diagnosis and the development of rehabilitative strategies.

Proposed Course: Now that antidromic stimulation has demonstrated a functional connection from movement-related cells in the frontal eye field to the superior colliculus, a survey will be undertaken to determine which classes of frontal eye field neurons project to the colliculus. In addition, orthdromic stimulation from the superior colliculus to the frontal eye fields and from the frontal eye fields to the superior colliculus will be used to analyze information flow between these two areas. The effect of eye and head position on saccades evoked from the frontal eye fields will also be studied, and methods developed for the electrical recording and stimulation from animals with unrestrained heads.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications:

Bruce CJ and Goldberg ME: The physiology of the frontal eye fields. Trends in Neurosci (in press).

Goldberg ME: Book review: Spatially Oriented Behavior. Hein A, and Jeannerod M, editors. Neuropsychologia 22:103, 1984.

Goldberg ME: Neuronal basis of visual attention. Neurosci Lett 14(Suppl):S139, 1983.

Bruce CJ and Goldberg ME: Primate frontal eye fields: Single neurons discharging before saccades. J Neurophysiol (in press).

Goldberg ME and Bruce CJ: Cortical activity associated with the orienting of visual attention. Vision Res (in press).

Heilman KM, Watson RT, Valenstein E, and Goldberg ME: Attention. In Handbook of Physiology. Section I: The Nervous System, Plum F, editor. Bethesda, American Physiological Society (in press).

Segraves MA and Goldberg ME: Initial orbital position affects the trajectories of large saccades evoked by electrical stimulation of the monkey superior colliculus. Soc Neurosci Abstr (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00047-06 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Processing in Brains following Cortical Ablation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael E. Goldberg	M.D.	Chief, NMS	LSR, NEI
Others:	Deng Shu-yi	M.D., Ph.D.	Visiting Fellow	LSR, NEI

COOPERATING UNITS *(if any)*
Laboratory of Neuropsychology, National Institute of Mental Health
(L. Ungerleider, M. Mishkin);
Department of Neurology, Georgetown University School of Medicine (M. Goldberg)

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Neuro-Ophthalmologic Mechanisms Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.1	0.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monkeys were prepared with unilateral frontal eye field ablations. They were then trained on a series of oculomotor tasks. They could easily perform visually guided saccadic eye movements but were impaired in learning to make eye movements to remembered points or eye movements in which the retinal location of the stimulus was different from the direction of eye movement necessary to acquire that stimulus' position.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Recent work in this laboratory has shown that the monkey frontal eye fields contain cells which signal the amplitude and direction of an impending rapid eye movement, or saccade. This activity occurs before visually guided saccades and other purposive saccades, but not before saccades made spontaneously in the dark without visual or auditory target or task requirement. Ongoing sensory stimulation is not necessary for this activity because it occurs before saccades made to the remembered position of a target in total darkness. Despite the presence of a presaccadic signal in the frontal eye fields, there has been little evidence that lesions of the frontal eye fields affect saccades. Since the superior colliculus also has cells that discharge before visually guided saccades, such simple eye movements could be controlled exclusively by the colliculus, and would not need a frontal eye field. The frontal eye fields would be necessary to provide the oculomotor signal for more complicated eye movement tasks. We decided to see if an animal with a lesion in the frontal eye field, and therefore relying predominantly on the superior colliculus for the generation of saccades, was capable of performing more difficult oculomotor tasks.

Methods Employed: Monkeys were implanted with magnetic search coils for the measurement of eye position. They also underwent unilateral surgical ablation of the frontal eye fields. The monkeys were trained to perform a number of simple visuomotor tasks, including visual fixation and visually guided saccades. They were also trained on two special tasks. In one (the gap task) they had to fixate a spot of light. While they fixated, a second spot appeared in the periphery and remained on for several hundred milliseconds. One hundred to six hundred milliseconds after the spot in the periphery disappeared the central fixation spot disappeared and the monkeys had to move their eyes to where the target had been. In the second task (the double jump task), the monkey began by fixating a central fixation point, which disappeared and two spots appeared sequentially, one for 100 to 125 msec, and the second for 50 msec. The monkey then had to move its eyes to where each of the stimuli had been, and was rewarded for making both of the two required saccades. This task created a dissonance betweeen the retinal location of the stimulus and the direction of the eye movement needed to acquire it. For example, the monkey might have to make a downward saccade to get to the location of a stimulus that only appeared upward on the retina.

Major Findings: Monkeys with unilateral frontal eye field lesions easily learned to fixate spots of light and make visually guided saccades. Monkeys that were trained on making saccades into the visual field ipsilateral to the lesion made accurate saccades immediately when the target was moved into the field contralateral to the lesions. The velocities of saccades into both fields were normal. The monkeys were then trained on the gap task with stimuli in the ipsilateral field, and learned to make accurate saccades to remembered targets quickly. They had great difficulty making accurate saccades to stimuli in the ipsilateral field when those stimuli had been missing for more than 100 msec. This difficulty lasted for nearly a month, but slowly recovered. After the deficit recovered the monkey's saccades into the ipsilateral field were always slower and less accurate than those into the contralateral field.

The lesioned monkeys also had great difficulty learning the double jump task. When the stimuli appeared contralateral the lesion but the second movement

was ipsilateral to the lesion the monkeys could easily make the second movement. However, when the stimuli appeared ipsilaterally but the second movement was contralateral the monkeys could make the movement. This implies that the critical factor for the frontal eye fields is not the retinal or spatial location of the stimulus but rather the direction of the eye movement necessary to acquire the stimulus.

One monkey learned the tasks before undergoing frontal eye field ablation. This animal did not lose the ability to perform the task after the surgery, although its performance was not as accurate as before the lesion. These results indicate that the frontal eye fields are more important for learning new and difficult oculomotor tasks than they are for the performance of simple or well-learned saccades.

Significance to Biomedical Research and the Program of the Institute: Understanding how the cerebral cortex guides eye movements is useful both as a model for the neural control of other more complicated motor behaviors and as a key to the interaction of the visual and the oculomotor systems. Development of more sophisticated methods for the demonstration of functional deficits will lead to the growth of new diagnostic, therapeutic, and rehabilitative strategies in patients with disease of the central visual and oculomotor systems.

Proposed Course: The effect on oculomotor performance of adding punctate electrolytic and chemical lesions of the superior colliculus in monkeys with unilateral striate ablations will be studied. Patients with unilateral frontal lesions will be studied using similar behavioral and analytic methods.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Disorders--Sensory Neuro-Ophthalmic Disorders)

Publications:

Deng S-Y, Segraves, MA, Ungerleider LN, Mishkin M, and Goldberg ME: Unilateral frontal eye field lesions degrade saccadic performance in the rhesus monkey. Soc Neurosci Abstr (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00153-02 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adaptive Regulation in Primate Oculomotor System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Frederick A. Miles	D.Phil	Chief, OCS	LSR, NEI
Others:	Kenji Kawano	M.D., Ph.D.	Visiting Scientist	LSR, NEI
	Lance Optican	Ph.D.	Senior Staff Fellow	LSR, NEI
	Reuben Gellman	Ph.D.	Visiting Fellow	LSR, NEI
	James Carl	M.D.	Staff Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Oculomotor Control Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.0	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiments were concerned with the initial ocular following responses to transient ramp movements of the visual scene in monkeys and humans. These tracking movements are important in the stabilization of gaze which is so necessary for good visual acuity. We had previously shown in monkeys that responses have very short latency (approximately 50 msec) and are transiently enhanced following saccadic eye movements. We have now found that this enhancement results from the visual stimulation elicited by the saccade sweeping the retina across the visual scene. When no such visual stimulation is produced, as when a vertical saccade is made while viewing vertical stripes, for example, then no enhancement results: saccadic eye movements per se are not sufficient to elicit the enhancement. Furthermore, the enhancement could be evoked in the absence of saccades by shifting the visual scene in a manner that mimicked the visual events occurring during saccades: the visual stimulation received during saccadic eye movements is entirely responsible for the transient post-saccadic enhancement of ocular following. Using sine wave grating patterns of various spatial frequencies (0.05-1 cycles/^{o}) drifting at various velocities (5-400^{o}/sec) to elicit ocular following revealed that the response latency was solely determined by the temporal frequency. This suggests that ocular following responses are triggered by local changes in luminance rather than movements of the overall pattern. Attempts to demonstrate inter-ocular transfer of enhancement were not successful: An optical arrangement that allowed the visual scene to sweep across one eye only during saccades did not enhance the ocular following responses elicited by movements seen only by the other eye. This is consistent with the view that the visual enhancement of ocular following is due to changes that occur in the visual system at an early stage in processing before information from the two eyes converges.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 80-EI-93

Objectives: Good visual acuity requires that retinal images be kept reasonably stable and one of the major problems confronting the oculomotor system is to keep the eyes still. One of the mechanisms that helps to achieve this operates by tracking any slippage of the retinal image. Since the usual visual scene is stationary, this ocular tracking system helps to "lock" the eyes in place. We had previously found that the monkey's ocular tracking responses to sudden, brief shifts of the visual scene were of unexpectedly short latency--commonly 50 msec or less with optimal stimuli--and were very sensitive to the prior occurrence of a saccadic eye movement. The initial ocular acceleration rate was always greatest to shifts occurring immediately after saccadic eye movements and diminished progressively as the post-saccadic interval increased, being reduced to less than 30% after 300 msec. The present experiments were concerned with the origin of this post-saccadic enhancement and, in particular, whether it resulted from the occurrence of a saccade per se, or from the associated visual stimulation due to the eye sweeping across the scene. A further objective was to characterize the spatial and temporal frequency dependence of these rapid ocular following responses using sine wave grating patterns as the visual stimulus. Preliminary attempts were also made to demonstrate similar phenomena in normal human subjects.

Methods Employed: Monkeys faced a screen subtending 85° horizontally and vertically, and their eye movements were recorded using the electromagnetic scleral search coil technique. Various patterns (random dots, and sine wave gratings of various spatial frequencies and orientations) were back-projected onto the screen by an x-y mirror galvanometer system under computer control. Initial ocular tracking responses to very brief movements of the scene were assessed using 100 msec test ramps delivered at various times after the animal's spontaneous saccades, randomizing both direction and speed (10-400°/sec). Tracking responses were never reinforced.

Eye movement recordings were also initiated in normal human subjects using a scleral search coil embedded in a silastic annulus that fits the eye like a contact lens. The visual stimuli were random dot patterns subtending about 80° and moving under computer control exactly as in the experiments on monkeys.

Major Findings: Experiments were concerned with the ocular tracking responses elicited by brief (100ms), unexpected, ramp movements of the visual scene. Though ramps were randomized for time of onset, direction and speed, and tracking was never reinforced, response latencies were invariably short, e.g., mean latency to 40°/s ramps was 51.5 $\pm$ 3.1(SD)ms (n=8 animals). However, as we previously reported, response amplitudes were strongly influenced by a prior saccade: initial eye acceleration was greatest when ramps began immediately after (spontaneous) saccades and declined roughly exponentially as the post-saccadic delay interval was increased (mean time constant, 73ms; mean asymptote, 30%; n=5). Subdividing responses according to the magnitude and/or direction of the antecedent saccade failed to reveal any consistent asymmetries: saccade parameters were irrelevant.

However, using sine wave grating patterns in place of the random dots, initial tracking responses were extremely sensitive to both the magnitude and the direction of the antecedent saccade: shifts of the visual scene following saccades in the direction of the stripes were associated with very poor initial ocular following responses, and the usual post-saccadic enhancement of such tracking was entirely absent. In fact, the enhancement of tracking was crucially dependent upon the antecedent saccade sweeping the eyes across the stripes. Thus, the occurrence of a saccade was not itself sufficient to enhance tracking responses: the post-saccadic enhancement of ocular following was due entirely to the visual stimulation produced by the saccade.

Using gratings of various spatial frequencies (F_S) moving at various velocities (V), clear short-latency tracking was seen only with spatial frequencies less than 1 cycle/^{o} and latency was solely dependent upon temporal frequency, F_T (= F_S*V): For temporal frequencies less than 10Hz, all data could be fitted (r=0.93) by the equation, latency (in ms) = $78F_T^{-0.185}$; for temporal frequencies greater than 10Hz, latencies levelled off at minima of 48-50ms, until responses began to fail entirely with temporal frequencies greater than 50Hz. These tracking responses are therefore initiated by mechanisms that do not respond to movements of the whole pattern but to small parts of it, e.g., doubling the speed at which the pattern drifts across the retina would have no effect on the latency if the spatial frequency were halved to preserve the same temporal frequency. This strongly suggests that tracking was triggered by local contrast changes rather than overall movement of the pattern.

Reductions in the contrast, C, of the gratings caused minor decrements in initial eye acceleration and moderate increments in latency, e.g., for 0.27 cycles/^{o} gratings moving at 60^o/s, reducing contrast from 0.5 to 0.01 reduced initial eye acceleration by only 10% while increasing latency by nearly 20ms and all data could be fitted (r=0.94) by the equation latency (in ms) = $47C^{-0.077}$. The selectivity for low F_S and relative insensitivity to contrast allow the system to tolerate considerable blur--a most useful property for a visual stabilization mechanism.

It was also possible to show that not only were saccadic eye movements alone not sufficient to enhance the initial ocular following response, but they were also not necessary. Initial ocular following responses could be enhanced by merely shifting the scene in a manner that simulated the visual events occurring during a saccade. These "simulated saccades" were achieved by moving the scene at 480^o/sec for periods of 20 msec (9.6^o amplitude shift): after such shifts, ocular following responses were transiently enhanced with a magnitude and time course very similar to that seen after saccadic eye movements. Thus, it was possible to mimic post-saccadic enhancement of ocular following by shifting the visual scene in a saccade-like way. This wave of increased excitability evoked by sudden changes in the retinal image resembles the so-called shift effect in the retina, in which the excitability of y-type retinal ganglion cells can be transiently enhanced by sudden changes that occur in the visual field far beyond the cells' normally defined receptive fields. This suggested that the enhancement might even originate in the retina. For this reason, we attempted to determine whether the enhancement of ocular following caused by saccadic shifts of the field showed inter-ocular transfer. Using a haploscope, the left eye was exposed to horizontal stripes and the right eye to vertical stripes. The vertical stripes seen by the right eye were then subjected to horizontal ramp movements in the wake of saccadic eye movements. Note that in these circumstances pure

vertical saccades result in visual stimulation of the left retina only, while pure horizontal saccades result in visual stimulation of the right retina only. Under these conditions, we found that the ocular following responses elicited by horizontal movement of the stripes in front of the right eye were not enhanced after vertical saccades. Thus, no interocular transfer of enhancement was found.

Preliminary recordings of ocular following responses in normal human subjects suggest a very different organization: (1) the latency is at least 20 msec longer; (2) the initial rate of eye acceleration for any given stimulus ramp was much lower--often an order of magnitude; (3) thus far, we have seen little to suggest that there is any post-saccadic or post-shift enhancement. It is possible that we have used inappropriate stimulus parameters, hence we do not yet entirely rule out the possibility of finding some type of enhancement phenomenon. More experiments are required.

Significance to Biomedical Research and the Program of the Institute: The present experiments have demonstrated a new visual enhancement phenomenon in the monkey's oculomotor system that may play a significant role in the visual stabilization of the eyes following saccadic displacements. Since clear vision requires good stablization of the eyes, these findings may provide new insights into an oculomotor mechanism that is important for good visual acuity. Preliminary observations failed to demonstrate a similar phenomenon in human subjects, but it is possible that this was due to technical shortcomings: the temporal parameters may be significantly different in man and more experiments need to be done to clarify this important issue.

Proposed Course: More extensive tests will be carried out in normal human subjects in an attempt to show the existence of transient visual enhancement of ocular following. In monkeys, attempts will be made to elicit the enhancement using a visual stimulus paradigm that more closely resembles that used to demonstrate the "shift effect" in the retina. Other concerns will be the receptive field, and temporal and spatial frequency dependence of the visual enhancement mechanism.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Miles FA: Sensing self-motion: Visual and vestibular mechanisms share the same frame of reference. Trends in Neurosci (in press).

Lisberger SG, Miles FA, and Zee DS: Signals used to compute errors in the monkey vestibulo-ocular reflex: possible role of the flocculus. J Neurophysiol (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00152-02 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visually Induced Adaptive Changes in Saccadic Innervation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lance M. Optican	Ph.D.	Senior Staff Fellow	LSR, NEI
Others:	Frederick A. Miles	D.Phil.	Chief, OCS	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Oculomotor Control Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Changes of fixation are made by rapid eye movements, called saccades, that have high velocities and abrupt endings. Disease or injury can interfere with the correct balance between the innervation and muscles and cause the eyes to drift after each saccade, interfering with clear vision. Earlier work in human patients and monkeys has shown that the post-saccadic ocular drift associated with peripheral ocular motor weakness can be corrected by a central neural mechanism within a few days. It has been proposed that this adaptive mechanism operates by adjusting the ratio of the phasic and tonic components (the pulse and step) of saccadic innervation.

The suppression of post-saccadic ocular drift was studied in normal monkeys by optically inducing retinal image slip after every saccade. It was found that retinal slip alone was sufficient to elicit post-saccadic drift suppression. It was also discovered that the time constant of the ocular drift's waveform depended on the time constant of the optically imposed image slip. Simulation of these results with a mathematical model of the saccadic system led to the conclusion that the saccadic innervation must consist of three parts, with a transition component (or slide) being added between the phasic and tonic components. Analysis of the model showed that three factors had to be controlled to achieve adaptive suppression of post-saccadic ocular drift: the gain of the step of innervation, and both the gain and the time constant of the slide of innervation.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 80-EI-93

Objectives: The objectives of this project are to understand the detailed nature of the neural innervation sent by the brain to the extraocular muscles to produce a saccade, and to determine how the adaptive mechanism adjusts this innervation to compensate for peripheral disorders. The goal is a mathematical model of the saccadic system that is sufficiently detailed that it can account for saccadic system performance in both normal and abnormal subjects.

Methods Employed: Eye movements in monkeys were monitored with the magnetic field/search coil technique and recorded by a computer. Monkeys faced a translucent screen subtending 100° in both the horizontal and vertical directions. Highly textured, colored images were projected onto the back of this screen and were moved by a mirror galvanometer system under computer control. Saccadic eye movements were recorded in the dark before, during and after the animal was exposed to an adaptation paradigm. In the adaptation paradigm the visual scene was made to drift exponentially after each spontaneous saccade made by the monkey.

Major Findings: Persistent post-saccadic retinal slip alone is sufficient to elicit a post-saccadic ocular drift. This ocular drift developed even when extraocular muscle proprioception and final retinal image position were normal. The ocular drift did not affect the rapid part of the saccade, as indicated by a normal main sequence of peak eye velocity vs. saccade amplitude.

The adaptive response developed with a time constant of about 35 hours, but it recovered with a time constant of about 4 hours. When animals were put in complete darkness for several days, the adapted response decayed very slowly (with a time constant of about 80 hours), indicating the long-term, plastic, nature of the adaptive changes. Adjustments to the amplitude of the rapid part of a saccade occurred independently over a shorter period, with a time constant of about 2 hours.

The time constant of the ocular drift was linearly related to the time constant of the drift of the adapting stimulus. This implies that saccadic innervation, and not the mechanics of the eyes and muscles, determines the drift waveform. Physiological studies have shown that the saccadic innervation is complex, consisting of several phases. The most basic description of this innervation has been as a pulse and step, with the pulse corresponding to the phasic part and the step corresponding to the tonic part of the innervation. Analysis of a mathematical model of the saccadic system showed that this simplified pulse-step representation could not explain our data.

We added an exponential decay, or slide, to represent the transition from the phasic to the tonic parts of the saccadic innervation. Simulation of this pulse-slide-step model accurately reproduced both the normal and adapted saccades of monkeys. The simulation showed that three factors must be controlled by the adaptive mechanism to suppress post-saccadic ocular drift: the gain of the step of innervation, and both the gain and the time constant of the slide of innervation.

Significance to Biomedical Research and the Program of the Institute: This work has advanced our understanding of those properties of the neural innervation which are important in making saccadic eye movements. This will influence all other work that attempts to study the neurophysiological basis of saccadic performance. The detailed description of the adaptive changes elicited by retinal slip also makes possible further progress in understanding other adaptive mechanisms, for while previous work has emphasized the adaptive changes of neural gain elements, this work shows that adaptive changes of the time constants of neural elements are also important. The quantification of the time course of the different phases (i.e., acquisition, recovery and decay) of the adaptation is also important clinically, since it can be used to distinguish between acute and chronic ocular motor disorders of both peripheral and central origins.

Proposed Course: This work suggests that retinal slip, a visual signal, is sufficient to elicit an adaptive change in the saccadic motor control system. A detailed mathematical model of the motor part of the saccadic system is now available, but it is not known how the visual system influences this model. The next step will be to attempt to find the necessary link between retinal image motion and long-term, plastic changes in the saccadic motor system.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Normal and Abnormal Development)

Publications:

Optican LM and Zee DS: A hypothetical explanation of congenital nystagmus. Biol Cybernetics 50:119, 1984.

Zee DS, Chu FC, Optican LM, Carl JR, and Reingold DB: Graphic analysis of paralytic strabismus with the Lancaster red-green test. Am J Ophthalmol 97:587, 1984.

Optican LM: Adaptive properties of the saccadic system. In Adaptive Mechanisms in Visual-Vestibular Interaction, Berthoz A and Melvill Jones G, editors. Elsevier Press (in press).

Miles FA, Optican LM, and Lisberger SG: An adaptive equalizer model of the primate vestibulo-ocular reflex. In Adaptive Mechanisms in Visual-Vestibular Interaction, Berthoz A and Melvill Jones G, editors. Elsevier Press (in press).

Zee DS and Optican LM: Studies of adaptation in human ocular motor disorder In Adaptive mechanisms in visual-vestibular interaction, Berthoz A and Melvill Jones G, editors. Elsevier Press (in press).

Zee DS and Optican LM: Mechanisms of ocular oscillations. In Tremor, Findley L and Capildeo R, editors. Macmillan (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 0045-06 LSR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Visuomotor Properties of Neurons in the Thalamus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David Lee Robinson	Ph.D.	Research Physiologist	LSR, NEI
Others:	Steven E. Petersen	Ph.D.	Staff Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Visuomotor Integration Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.0	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are currently studying how parts of the visual system deal with different types of stimulus movement. Neurons in the inferior pulvinar and alpha division of the lateral pulvinar respond to stimuli moving while animals hold their eyes stationary. Many of these cells respond over a wide range of velocities from a few degrees per second to several hundred degrees per second. Many cells in both of these areas do not respond to comparable motion of the same stimulus when it is generated by an eye movement. This ability to distinguish between real and self-induced stimulus motion persists when tested in a darkened environment. These observations suggest that the lack of response with an eye movement is not due to visual factors and may be generated by a corollary discharge. Cells with these properties are also found in the superior colliculus but not visual cortex, both of which project to the pulvinar. Hence the collicular pathway may mediate this process. These studies suggest that one function of this part of the visual system is in dealing with non-visual influences on the processing of visual image motion.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The purpose of this project was to study how the nuclei of the pulvinar deal with different types of stimulus motion. Images moving in the external world excite a series of retinal receptors and are perceived as real image motion. Similarly, the motion of the eye causes sequential excitation of the retinal receptors, but this is not perceived as image motion. It has long been unclear how the brain distinguishes between these different types of visual stimulation. Since there are visually responsive neurons in several subregions of the pulvinar, we attempted to determine their responsiveness to stimulus motion, if they distinguish between these types of stimulation, and how they accomplish this distinction.

Methods Employed: Animals were trained to fixate a spot of light projected on a tangent screen and release a bar when it dimmed. We monitored eye position with a scleral search coil, and inappropriate eye movements terminated a trial. During periods of fixation we flashed or moved lights on the tangent screen to determine the location of a cell's visual receptive field and the response to different velocities of stimulus motion. To test for self-induced stimulus motion we had the monkey fixate the same spot of light; then we turned on a second spot of light and simultaneously turned off the fixation light. The monkey made a saccadic eye movement to this new target in order to detect its dimming. On a percentage of the eye movement trials, we positioned a stationary stimulus so that the eye movement generated by the animal swept the receptive field over the stationary stimulus. Similar tests were conducted with the background illumination turned off. After completion of many such experiments the brains were examined histologically for identification of the recording sites. Presentation of all targets, stimuli, and rewards as well as control of the animals' eye position was accomplished with an on-line digital computer.

Major Findings: Initially we studied the response of pulvinar cells to stimuli moving at different velocities and in different behavioral conditions. We tested neurons in two different regions of the pulvinar: a retinotopically mapped inferior area and a retinotopically organized region of the lateral pulvinar (the alpha subdivision). Most cells in both regions respond to all directions of stimulus movement, but some respond only to a limited range of directions. Many cells in both of these areas are excited by stimuli moved across their visual receptive fields while monkeys hold their eyes stationary. These cells can respond to stimuli moving over a very wide range of velocities from just a few degrees per second to several hundred degrees per second. Thus, cells in this part of the brain are not selective for stimulus velocity as are those in cortical areas.

Although most pulvinar cells discharge to stimuli which move while a monkey holds its eye stationary, many of these same cells do not respond to comparable stimulus motion which is generated by the animal making an eye movement. If a stimulus is placed next to a cell's visual receptive field so that an eye movement sweeps the receptive field over the stationary stimulus, many cells in both parts of the pulvinar do not respond as expected. These cells must receive some input which prevents them from responding to images which move during an eye movement. There are also cells in both of these areas that respond to both types of retinal stimulus motion.

We next ran a set of experiments to determine the factors that contribute to the inability of some pulvinar cells to respond to stimulus motion during eye movements. We conducted the same stimulus movement and eye movement tests in a darkened environment, and these cells reacted the same way. This suggests that the lack of response with an eye movement is not visually-mediated; the effect persists when visual influences are eliminated. Therefore, a proprioceptive signal from the extraocular muscles or a corollary discharge from the central nervous system reduce the visual responsivity of these cells with eye movements; either is a likely candidate for the mechanism.

Similar experiments have been conducted with the cells in the superior colliculus and visual cortex. All of the cells in visual cortex respond with both real and self-induced stimulus motion, but there are many cells in the superior colliculus that are excited by real motion and not to self-induced motion. The region of the superior colliculus that contains these cells projects to the inferior mapped region of the pulvinar. The distinction of real from self-induced movement may be transmitted to the thalamus by way of this ascending pathway. Other experiments have demonstrated that visual properties found in this part of the pulvinar are mediated by visual cortex and not by the superior colliculus. Our studies provide evidence for a functional contribution of the ascending colliculo-pulvinar pathway. This pathway has been proposed as a major route in the extra-geniculostriate visual system.

Significance to Biomedical Research and the Program of the Institute: One of the major problems in understanding the central mechanisms of vision to determine what elements of visual behavior are controlled by what parts of the brain. Once we have determined how the elements are segregated in the brain, we can better determine defects in visual performance in people with damage to their central nervous system. The work reported here helps understand the visual functions performed by certain regions of the brain. This knowledge will be valuable to the clinician in localizing damaged or deficient areas of the brain. In addition it will be helpful in developing techniques for training patients to utilize other parts of their brain to compensate for lost capacities.

Proposed Course: Future experiments will attempt to determine additional characteristics of the cells which do not respond with self-induced stimulus motion and establish the mechanism of this effect. We will test to see if the effect is a threshold elevation or a total blanking of visual signals. Experiments will determine if the process is operational for all directions of motion or just some. We will also test the visual excitablity of these cells after an eye movement to calculate the time-course of the process. Finally, we will study these cells during the temporary inactivation of the superior colliculus to establish if this is the source of the effect.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Petersen SE and Robinson DL: Responses of pulvinar neurons to real and self-induced stimulus movement. Invest Ophthalmol Vis Sci 25:34, 1984.

Robinson DL and Petersen SE: Posterior parietal cortex of the awake monkey: Visual responses and their modulation by behavior. In Integration at Basic and Cortical Levels, Reinoso-Suarez F, editor. New York, IBRO Monograph Series (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00191-01 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of the Pulvinar and Parietal Cortex in Visual Attention

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David Lee Robinson	Ph.D.	Research Physiologist	LSR, NEI
Others:	Steven E. Petersen	Ph.D.	Staff Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Visuomotor Integration Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying a subdivision of the pulvinar to determine its role in selective visual attention. Monkeys and humans were trained to fixate on a spot of light and respond to the onset of a peripheral light. Reaction times for both species were faster when the target was preceded by a cue correctly indicating the location of the target; reaction times were slowed by invalid cues. These data suggest that the cue shifts attention to one side and speeds or slows responses with valid or invalid cues, respectively. Since our electrophysiological recordings suggested that the beta subdivision of the lateral pulvinar is related to visual attention, we injected drugs into this area in two monkeys in an attempt to change the monkeys' attentional behavior. GABA is a transmitter in this part of the brain, and we used drugs which either increased or decreased GABA levels. Increased levels of GABA were associated with slowed attention shifts to the contralateral side; decreased GABA levels were associated with speeded attentional shifts. These studies support a role for this area of the brain in selective visual attention.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 80-EI-93

Objectives: Stimuli continually strike the retina, but only a fraction are used for behavior. One of the processes active in the behavioral use of a visual image is the shift of attention. The way in which stimuli evoke a shift of attention and the definition of areas of the brain related to attentional shifts have been issues of interest to psychologists, neuroscientists and clinicians for decades. The purpose of the experiments described here was to establish behavioral techniques which would lead to a measurable control of attention in both man and monkey. Once the techniques were developed, then it would be possible to attempt to manipulate brain areas in experimental animals and affect attentional behavior.

Our previous electrophysiological studies have shown that neurons in the beta subdivision of the lateral pulvinar have properties suggestive of an attentional function. The neuronal properties are similar to those of the posterior parietal cortex, a region that has been implicated in attentional behavior from both clinical and physiological data. We attempted to pharmacologically manipulate this part of the thalamus in an attempt to change the monkeys' attentional behavior. GABA is known to be a transmitter in the pulvinar, so drugs were injected which modify levels of GABA and the resulting changes in the monkeys' reaction times were measured. Also the same behavioral techniques would allow for testing of humans with damage of the brain thought to be related to attention. Both of these approaches would help localize structures related to attention and help define the processes and mechanisms underlying the shift of attention.

Methods Employed: For these experiments on visual attention, monkeys were trained to fixate on a spot of light on a tangent screen and depress a bar as soon as a target light was flashed in the periphery. Their eye position was monitored with a scleral search coil, and inappropriate eye movements terminated trials. Reaction times were measured for the bar presses. After this initial training, the onset of the target spot was preceded by a large cue light which correctly indicated the location of the subsequent target. On a small fraction of trials the cue invalidly indicated the side of the target. After baseline data were collected from each animal for several variants of this task, microelectrode penetrations were made to locate the beta subdivision of the lateral pulvinar. A guide tube was implanted over this part of the pulvinar to allow the localized injection of drugs. We then injected bicuculline, a GABA-antagonist which elevates GABA levels, or muscimol, a GABA-agonist which lowers GABA levels. After injections of an individual drug, we tested the monkeys with several runs of validly and invalidly cued trials and recorded reaction times. After completion of many such experiments, the brains were examined histologically for identification of the injection and recording sites. Presentation of all cues, lights, and rewards as well as control of the animals' eye position was accomplished with an on-line digital computer.

Major Findings: The first set of experiments were directed toward measuring attentional behavior in man and monkeys. Our initial experiments determined the standard reaction times of humans and monkeys to the onset of a peripheral target

presented while the subject fixated. Subsequent tests showed that these reaction times were faster when the location of the target was preceded by a cue stimulus which correctly (validly) indicated the location of the target. Conversely, reaction times were slowed if the cue incorrectly (invalidly) indicated the location of the target. Data also show that valid and invalid cues give faster and slower reaction times respectively, when compared to: (1) interspersed trials where a full-field cue is flashed so that there is no information about spatial location, and (2) separate runs in which there is no cue presented. Qualitatively, our data for man and monkey were quite similar, but the differences in reaction times for valid and invalid cues were larger and behavior was generally less variable for man than monkey.

There are four possible test conditions for each of the drugs to affect, valid and invalid trials on the ipsilateral and contralateral sides. The specific results in these situations show the influence of the drugs on attentive behavior. When both cue and target were in the visual field ipsilateral to the drug injection (bicuculline or muscimol), reaction times were unaffected. Thus the drug effects were confined to contralateral visual field. When both cue and target were in the contralateral field, reaction times in control and bicuculline conditions were similar; muscimol treatment slowed responding. Muscimol had a slowing effect but bicuculline could not facilitate the already fast response. For invalidly cued trials, the drugs gave opposing results. Following bicuculline injection, reaction times were faster with the cue in the ipsilateral field, slower with a contralateral cue. Since bicuculline facilitated attention to the contralateral side, this speeded responses with invalid, ipsilateral cues and slowed them with invalid, contralateral cues. Following muscimol injection, reaction times were slower with the invalid cue in the ipsilateral field, faster with a contralateral, invalid cue. Since muscimol slowed attention shifts to the contralateral side, this slowed responses with invalid, ipsilateral cues and speeded responses with invalid, contralateral cues.

Several general observations summarize the effects of drug injection on the performance of this task. The effects of the drugs were limited to the contralateral visual field. When both the cue and target were in the ipsilateral visual field, neither of the drugs influenced the behavior. Whenever the cue or target was in the contralateral field the two drugs had opposing effects. This is consistent with their opposing pharmacology. The drug effects were primarily related to the cue and were most apparent on invalidly cued trials, which is consistent with the hypothesis that drug injection into the pulvinar primarily affects spatial attention.

We have recently begun collaborative studies with Dr. Jon Currie of the Neuro-ophthalmology Section, NEI, and have tested one patient with a lesion of his non-dominant parietal cortex. In our attention task, this individual has an interesting set of responses. For validly cued trials on either side, his reaction times are relatively normal, but there is a slight tendency for him to respond slower for targets in his affected field than in his unaffected field. He also has normal responses in the invalid condition where the cue is in his affected field and the target is in his unaffected field. However, he has much longer reaction times in the situation where the cue comes on in his good field and the target appears in his affected field. In a situation where his attention has been shifted into his good field, he has great difficulty shifting his attention back into his deficient field. These findings replicate the results of Pos-

ner on parietally damaged humans, and are consistent with the association of parietal cortex with attentional behavior.

Significance to Biomedical Research and the Program of the Institute: Different parts of the brain are involved in different functions. One of the problems facing the clinical neuro-ophthalmologist is localizing the site of damage of an individual with some central brain lesion. As we begin to understand the specific function of certain brain areas, we can develop tests which will require the use of these brain areas. Patients with damage to such areas will have clear difficulties performing such tasks. The approaches outlined in this project could provide valuable tools for diagnosing specific brain lesions. An understanding of normal brain function allows one to see how the brain changes in the face of damage to other parts. Furthermore, when we understand the function of a certain area and the neural mechanisms underlying that function, then we will be in a better position to therapeutically modify these processes.

Proposed Course: Future experiments will attempt to determine the effect of total inactivation of the pulvinar on attentional performance. This will be accomplished by injection of local anesthetics or use of a cooling probe. Additional studies will utilize these approaches to study cortical area 7 which is also important in visual attention. The goal of these experiments is to separate the functional contributions of these two areas to selective visual attention. Other studies will test humans with parietal cortex, frontal cortex, and thalamic lesions to assess their attentional behavior and correlate the results with hypotheses derived from experimental animal work.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Robinson DL: On the parietal cortex of monkey and man. Quart Rev Biol 59:98, 1984.

Robinson DL, Morris JD, and Petersen SE: Cued visual behavior and the pulvinar of the awake macaque. Invest Ophthalmol Vis Sci 25:33, 1984.

Robinson DL and Petersen SE: The neurobiology of attention. In Brain and Mind: Dialogues between Cognitive Psychology and Neuroscience. LeDoux J and Hirst W, editors. New York, Cambridge University Press (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00102-05 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Substantia Nigra in the Initiation of Eye Movements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Wurtz	Ph.D.	Chief	LSR, NEI
Others:	James R. Carl	M.D.	Clinical Fellow	LSR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Sensorimotor Research

SECTION
Visuomotor Integration Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The superior colliculus is a key center related to the initiation of rapid or saccadic eye movements, and one of the major influences acting on it is from the pars reticulata of substantia nigra. This segment of the substantia nigra is one of the two major output pathways of the basal ganglia. Our previous experiments have suggested that this pathway produces a tonic inhibition on the superior colliculus which is released at the time of saccade initiation. In the first experiment described, we tested this hypothesis by injecting a transmitter agonist γ-amino butyric acid (GABA), into the substantia nigra of an awake, behaving monkey and determined the effect on the monkey's ability to initiate saccadic eye movements. The injections produced irrepressible saccades to the part of the visual field served by substantia nigra. The monkey had greater difficulty making saccades to remembered targets rather than to visual targets. The results of these experiments are most easily interpreted as an increase of inhibition acting on the substantia nigra, a decrease in rate of discharges of substantia nigra cells, and a release of the superior colliculus from tonic inhibition. In a second set of experiments, the eye movements in patients with Parkinson's disease were examined since this disease affects the basal ganglia and might be expected to change the output related to the generation of saccadic eye movements. We found that the patients, like the monkeys, had greater difficulty making saccades to a remembered location than to a visual target. While the final eye position was nearly the same in both conditions, the velocity of the saccades to the remembered location was markedly altered, particularly during deceleration. This result, combined with the relatively normal saccades made to visible targets, suggests that in man, as in monkey, the basal ganglia are an important influence on saccadic control, particularly to remembered targets. In Parkinson's disease, the tonic inhibition of the substantia nigra on the superior colliculus presumably cannot be completely released to allow a normal saccade.

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 84-EI-22

Objectives: In studies on the superior colliculus in awake behaving monkeys, we have shown that this structure is the key brainstem center related to the initiation of rapid or saccadic eye movements. One of the major influences acting on the cells in the superior colliculus that discharge before the initiation of the saccadic eye movements is from the pars reticulata of the substantia nigra. This segment of the substantia nigra is one of the two major output pathways from the basal ganglia, a diencephalic region related to the initiation of movement. Our previous studies on the activity of cells in the substantia nigra of the monkey revealed that the discharge of many of these cells occurs in relation to the initiation of saccadic eye movements made to visual targets or to remembered targets. The discharge rates of these substantia nigra neurons are high and their response preceding saccadic eye movements to the contralateral visual field is a pause in this high rate of discharge. Since the action of these cells on the superior colliculus is likely to be inhibitory, this pause should act to release the cells in the superior colliculus from tonic inhibition and permit or facilitate the initiation of eye movements. We previously demonstrated that such a mechanism of action of the substantia nigra on the superior colliculus is likely by injecting transmitter agonists or antagonists of substantia nigra transmitter into the superior colliculus. Taken together, these previous experiments show that this output pathway of the basal ganglia produces a tonic inhibition on the superior colliculus which is released at the time of saccade initiation. Furthermore, the activity of cells and the effect of blocking the connection between the substantia nigra and the superior colliculus indicates that the substantia nigra is particularly related to the initiation of saccades made to remembered targets in the absence of direct visual control.

The first set of experiments described in this report examines further the role of the substantia nigra in the initiation of saccades to visual targets and to remembered targets. These investigations relied on the fact that the substantia nigra cells themselves are inhibited by)-amino butyric acid (GABA). Injection of a GABA agonist, muscimol, into the substantia nigra should increase the inhibition acting on the substantia nigra and should therefore reduce its inhibition on the subsequent processing in the superior colliculus. We examined the effect of this inactivation of the substantia nigra on the monkeys ability to make saccadic eye movements to visual and remembered targets.

The second set of experiments described utilized the information derived from the experiments on monkeys to test the deficit in human patients whose disease process might alter the activity of the substantia nigra pars reticulata. Patients examined had Parkinson's disease which affects the basal ganglia, and we investigated to what extent this might be revealed in the output pathway of the basal ganglia related to the generation of saccadic eye movements. Specifically, we were interested to see whether eye movements made to a visual or to a remembered target might be different and whether the dynamics of the saccade, such as velocity, might be different in these patients as compared to normals.

Methods Employed: In the experiments performed on monkeys we used a behavioral paradigm that required the monkeys to make saccades to visual targets

and to the location of targets that had to be remembered. The methods used in training the monkeys for these tasks have been described in previous annual reports. Under general anesthesia the monkeys had devices implanted for head restraint, for eye movement recording, and for single cell recording. During the experiments we used the magnetic search coil technique to record eye movements. Records of both cellular activity and eye movements were recorded using an on-line digital computer which also controlled the monkeys behavior. After the substantia nigra pars reticulata was located using single cell recording, we were able to insert a small guide tube into the brain which allowed injection of a GABA agonist (muscimol). Our procedure was to record the saccades made to visual targets and to remembered targets, inject muscimol into the substantia nigra, and then record the monkeys ability to make these eye movements after the injection.

For the experiments involving human patients, the same behavioral paradigm for saccades to visual targets and to remembered targets were used. The patient sat on a chair within the magnetic field and eye movements were recorded using the magnetic search coil technique which used a search coil embedded in a contact lens placed in one eye. The accuracy and dynamics of the saccadic eye movements were recorded.

Major Findings: The salient effect of the injection of muscimol into the substantia nigra pars reticulata was that the monkey made irrepressible saccades towards the contralateral visual fields where cells in the substantia nigra at the injection site had their movement fields. These saccadic jerks occurred when the monkeys attempted to maintain visual fixation or instead of saccades to visual targets or to remembered targets. The saccadic jerks also occurred during periods of spontaneous eye movements.

Saccades to remembered targets were more vulnerable to these saccadic intrusions than were saccades to visual targets. When the effects of muscimol were mild shortly after injection, the latencies for the saccades to remembered targets decreased for saccades towards the contralateral visual field, but increased for saccades to the ipsilateral visual field. An increase in the peak velocity of saccades was also seen: the velocity of saccades to remembered targets after the muscimol injection was raised nearly to the higher velocity that was typical of saccades to visual targets.

Several hours after the injection of the muscimol into the substantia nigra the monkey was frequently unable to make saccades to the ipsilateral visual field but continued to make irrepressible saccades to the contralateral visual field. On the day following the injection in the substantia nigra the effects were not detectable; the lesion was an entirely reversible one.

The results of these experiments are most easily interpreted as an increase of inhibition acting on the substantia nigra, a decrease in rate of discharge of substantia nigra cells, and a release of the superior colliculus from tonic inhibition. This release from inhibition leads to the irrepressible saccades.

Eye movement recordings were made on patients with hemiparkinsonism, with the right vs. left sides compared to reduce the effects of age, motivation, and mental status; these factors were the same for both sides. Saccades made to a visible target were relatively mildly affected, while those to a remembered target location were more abnormal. The latency of the saccades and the final eye position accuracy were not altered in a consistent fashion when the saccades were

to remembered targets rather than visual targets. The velocity of the saccades was, however, markedly changed, especially in the deceleration phase of the eye movement. This suggests that the tonic inhibitory effect of the substantia nigra on the superior colliculus could not be reduced appropriately, leading to the marked prolongation of saccades to remembered targets.

Significance to Biomedical Research and the Program of the Institute: The experiments on monkeys have revealed functions of the basal ganglia related to the initiation of saccadic eye movements. The activity of cells in the output of this pathway and the elimination of this pathway by transmitter blockers have led to a consistent picture of inhibition of one brain structure upon another, the substantia nigra upon the superior colliculus. This in turn allows the development of hypotheses about how structures in the human brain might function in normal behavior and in disease. We have been able to begin to test these hypotheses in human patients this year by beginning an analysis of the deficits in eye movements observed in Parkinson's disease. Thus the knowledge obtained from physiological, anatomical, and behavioral experiments in monkeys can be tested by analyzing the visuomotor behavior of man.

Proposed Course: Further experiments will concentrate on the characteristics of cells related to eye movements in the substantia nigra. A quantitative analysis of the deficit in human patients will also be undertaken.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Wurtz RH and Hikosaka O: Deficits in eye movements after injections of GABA-related drugs in monkey superior colliculus. Soc Neurosci Abstr 9:750, 1983.

Hikosaka O and Wurtz RH: Modification of saccadic eye movements by GABA-related substances I. Effect of muscimol and bicuculline in the monkey superior colliculus. J Neurophysiol (in press).

Hikosaka O and Wurtz RH: Modification of saccadic eye movements by GABA-related substances II. Effects of muscimol in the monkey substantia nigra pars reticulata. J Neurophysiol (in press).

Evarts EV, Kimura M, Wurtz RH, and Hikosaka O: Behavioral correlates of activity in basal ganglia neurons. Trends in Neurosci (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00109-04 LSR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Motion Processing in the Primate Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert H. Wurtz	Ph.D.	Chief	LSR, NEI
Others:	William T. Newsome	Ph.D.	Staff Fellow	LSR, NEI
	Akichika Mikami	M.D., Ph.D.	Visiting Scientist	LSR, NEI
	Max R. Dursteler	M.D.	Guest Researcher	LSR, NEI

COOPERATING UNITS *(if any)*

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SECTION
Visuomotor Integration Section

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	3.2	1.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Visual information processing in the primate cerebral cortex begins in the striate cortex and continues into extrastriate areas. One of these areas, the middle temporal area (MT), has a high proportion of cells that respond to stimuli moving within a restricted range of directions and velocities. Our work on this area in preceding years has concentrated on the visual and visual-motor properties of cells within this area including their role in initiation of slow pursuit eye movements made to moving visual targets. This year we have investigated the contribution of these cells to the perception of visual motion by using a visual illusion of motion. A sequence of stationary lights separated by appropriate spatial and temporal intervals gives rise to a vivid perception of motion, usually referred to as the phi-phenomena. We compared the threshold for a clear impression of motion in humans with the threshold at which directional selectivity became evident in striate cortex and MT cells. When we compared the human performance to that of a composite of the physiological responses, we found that human psychophysical performance corresponded to the best physiological response at all speeds. Which area, MT or striate cortex, corresponded more closely to the psychophysics, however, varied with the speed. At low speeds (2^{o}/sec), humans perceive motion for temporal intervals which in general did not sustain directional selective response in MT neurons but did in striate neurons. On the other hand, at high speeds (32^{o}/sec), perceived motion was more comparable to the response of MT cells. The results support the idea that directional selective cortical neurons are possible substrates for perception of apparent motion.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Protocol Number: 80-EI-93

Objectives: Visual information processing in the primate cerebral cortex begins in the striate cortex and continues in extrastriate visual areas. One of these areas, the middle temporal area (MT), has a high proportion of cells that respond to stimuli moving within a restricted range of directions and velocities. Our work on this area in preceding years has concentrated on the visual and visual-motor properties of cells within MT studied in awake behaving monkeys. In particular, we have investigated the role of MT on functions dependent upon the analysis of visual motion such as the initiation of slow pursuit eye movement made to moving visual targets. This year we have completed a study of the contribution of MT to another function: the perception of visual motion. We have used an illusion of apparent motion to study the responses of single neurons in area MT, and have compared this to the perception of such stimuli in humans.

Stimuli which give rise to an illusory perception typically contain only certain features of the stimuli which normally result in that perception, and by manipulating these features, we can determine the stimulus attributes that are critical for perception. One such illusion related to motion is referred to as the phi phenomenon: a sequence of stationary lights separated by appropriate spatial and temporal intervals gives rise to a vivid perception of motion. In our experiments we determined whether temporal intervals between flashed stimuli that give rise to the perception of apparent motion in humans are similar to the intervals which result in motion-like responses in directionally selective MT neurons. In addition, we have performed the physiological experiments on directionally selective neurons in striate cortex to see whether the responses of MT neurons differ significantly from the primary source of their input.

In our experiments the visual stimuli were trains of stationary flashes which traversed about ten degrees of the visual field. As the frequency of the flashes increased, a threshold for a clear impression of motion was reached for human observers. We compared this threshold in humans with the threshold for single cells recorded in the monkey at which directional selectivity became evident. At low flash frequencies, most direction selective neurons responded with a transient burst of spikes for each individual flash whether the sequence was in the preferred or the null direction for the neuron. However, at higher flash frequencies the neurons become directionally selective: the response continued to be modulated with each flash when the stimuli were displaced in the preferred direction, but an inhibitory mechanism repressed responses in the null direction.

Methods Employed: We recorded the responses of single neurons in awake behaving rhesus monkeys who were trained on a visual fixation task. The methods of recording single cells and eye movements have been reported previously. When a single neuron in area MT was isolated, we first determined its direction and speed selectivity using smoothly moving stimuli, and the optimal direction and the optimal speed were determined quantitatively. We then studied its responses to sequentially flashed sequences of stimuli which traversed the entire receptive field in the preferred and null directions. The strobe rate was varied randomly from trial to trial and eight presentations were usually obtained for each combination of spatial and temporal intervals. From this information a direction

index was computed (1 - response in the null direction / response in the preferred direction) which became larger as the directionality became more prominent. Our equivalent stimulus speeds were between 2 and 32°/sec. We determined the maximum temporal interval at which a criterion direction index was reached, and this criteria ranged from 0.5 to 0.9.

The psychophysical experiments conducted with humans used the same apparatus, with the same visual stimuli, under the same conditions of ambient illumination. The subject sat in a chair and faced the tangent screen with a chin rest provided for constant head position. The area of the visual field over which stroboscopic stimuli passed was set at an eccentricity of 5° to correspond generally to the eccentricity at which cells were recorded. The subjects task was to determine when the stimulus changed from jerky to smooth motion. We used the staircase method of threshold measurement for each stimulus presentation. Trials within a given block were presented at a constant equivalent speed. Again, speeds used were from 2 to 32°/sec.

Major Findings: For the neurons in area MT, we found that the transition from non-directional response to directional response was rather abrupt. Thus the arbitrary selection of a direction index value did not grossly disturb the results. The major finding was that the interstimulus interval at which a transition occurred varied with equivalent speed: smaller temporal intervals were generally necessary to ellicit direction selective responses at higher equivalent speeds. This procedure was repeated for neurons in the striate cortex. The striate neurons, despite having smaller receptive fields than MT neurons of similar eccentricity, exhibited directionally selective behavior for larger interstimulus intervals than did MT neurons at low (1-2°/sec) equivalent speeds. In contrast, MT neurons performed better at high (16-32°/sec) equivalent speeds. Performance was similar in the two areas at intermediate (4-8°/sec) speeds.

For human subjects, as for the single cells, the interstimulus interval at which the subjects saw smooth motion decreased as the equivalent speed of the stimulus increased. We then compared the human performance to that of a composite of the physiological response. We found that human psychophysical performance corresponded to the best physiological response at all speeds. Which area, MT or striate cortex, corresponded more closely to the human psychophysical response, however, varied with the speed. Thus at 2°/sec, humans perceived motion for temporal intervals which in general did not substain directional selective responses in MT neurons, but did in striate cortex neurons. At 32°/sec, the relationship was reversed: the observers perceived motion for interstimulus intervals which yielded direction selective responses in MT but failed to sustain direction selectivity in striate cortex.

These results support the notion that directionally selective cortical neurons are a neural substrate for perception of apparent motion, and they suggest that the neural processing of motion is distributed among cortical visual areas depending upon the speed of motion. In particular, MT neurons have the properties that would be necessary to support the perception of motion at relatively high stimulus speeds while striate cortex neurons are more likely to process information at low stimulus speeds.

Significance to Biomedical Research and the Program of the Institute: Some success has been obtained in comparing the activity of neurons at higher levels of the nervous system with visual processing leading to the generation of eye

movements, including many experiments performed in the Laboratory of Sensorimotor Research. However, the relation of such higher processing to visual perception is much less secure. This experiment attempts to relate specific regions of cerebral cortex to an identified perceptual function. In so doing, the hope of these experiments is to further identify the regions or circuits within the brain that are related to specific visual-motor functions, both oculomotor or perceptual.

Proposed Course: Future work will concentrate on the analysis of neurons in areas adjacent to MT in which further visual processing of motion presumably occurs.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Structure and Function--Behavior)

Publications:

Newsome WT, Wurtz RH, Dursteler MR, and Mikami A: Deficits in pursuit eye movements following chemical lesions of motion-related visual areas in the superior temporal sulcus of the macaque monkey. Soc Neurosci Abstr 9:154, 1983.

Newsome WT, Wurtz RH, Dursteler MR, and Mikami A: Deficits in visual motion processing following ibotenic acid lesions of extrastriate area MT of the macaque monkey. J Neurosci (in press).

LABORATORY OF VISION RESEARCH

ANNUAL REPORT
NATIONAL EYE INSTITUTE
October 1, 1983 - September 30, 1984

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH

Gerald J. Chader, Ph.D.

The mission of the Laboratory of Vision Research is to study normal and pathological processes in ocular tissues, with the primary emphasis on lens and retina. Using mainly biochemical techniques, LVR investigators elucidate and define important normal processes in the functioning of the tissues. In conjunction with this, disease processes are studied in an attempt to understand the etiology of the dysfunction. Animal models of human diseases are used extensively, although, where possible, both normal and affected human tissues are preferentially used.

Fiscal Year 1984 has been a time of major consolidation for the LVR with the loss of several investigators including Dr. Toichiro Kuwabara, Dr. Gerald Robison, who are now part of the Laboratory of Ocular Pathology, and Dr. Ralph Nelson, who is now in NINCDS. In spite of this, the productivity of the laboratory has been extremely high as attested to by the large number of excellent scientific papers published by lab members in the last 12 months. Following are highlights of some of the research conducted by members of the LVR during the year:

1. Section on Lens and Cataract: This group of investigators, under the leadership of Dr. Jin Kinoshita, remains the single most productive group in the field of lens research. Dr. Samuel Zigler has continued to study lenticular aging and cataractogenesis and has supplied important information implicating activated oxygen and lipid peroxides in the formation of some cataracts. Dr. Richard Bodaness has also uncovered a good deal of evidence linking oxidative changes with cataract formation. It appears from his work that hydrogen peroxide in the aqueous humor could be a potent precursor of oxidative agents that are toxic to the lens.

Dr. Paul Russell has studied several aspects of lens development and the role that glycolytic enzymes may play in cataractogenesis. Dr. Donita Garland has focused on the enzymes called protein kinases in the lens and has shown that many of the most important proteins of the lens can be phosphorylated by these enzymes, potentially modifying their structure and/or function.

Dr. Peter Kador has expanded his study of the formation and control of diabetic and galactosemic cataracts. In particular, the roles of the enzyme aldose reductase (AR) and of AR inhibitors in diabetes are being elucidated. It has been found that AR inhibitors are effective in retarding the basement membrane thickening that occurs in the capillaries of galactosemic rats. Thus, it seems that the AR enzyme plays a role in diabetes and that AR inhibitors could ultimately be useful in controlling the manifestations of the disease.

2. Section on Cell Biology: Dr. Paul O'Brien has made a most interesting basic science finding concerning the visual protein rhodopsin. He has found that rhodopsin can be acylated using the fatty acid, palmitate. The mechanism

appears to proceed via a palmitoyl CoA intermediate. The significance of this process has yet to be determined, but palmitate binding in other systems has been implicated in membrane fusion and transport.

Dr. Barbara Battelle has continued her pioneering study on the efferent innervation of the retina. She has accumulated a good deal of data demonstrating that efferent fibers have a major influence on photoreceptor cell biochemistry. This could indicate new directions in the study of the circadian rhythm process and possibly also in the study of some retinal diseases. Dr. Donald Puro also is interested in factors controlling neurobiological function of the retina, particularly with reference to agents controlling functional maturation. Substances such as dopamine, insulin, and the glucocorticoid hormones have been found to markedly affect the development of retinal neurons in culture. A direct application of these findings has been made in vivo since steroid hormones have been found to alter cholinergic neuronal development in the fetal rat retina.

3. Section on Retinal Metabolism: The members of this section are interested in elucidating normal and disease processes in the retina and the pigment epithelium. An excellent example of this is found in the work of Dr. Barbara Wiggert and her collaborators. This year has seen the culmination of several years work by Dr. Wiggert and Dr. Michael Redmond in identifying, purifying, and characterizing a new extra-cellular protein of the retinal interphotoreceptor matrix, the interphotoreceptor retinoid-binding protein (IRBP). This protein could very well be the major vehicle for retinoid transport between the retina and the pigment epithelium. Studies on a human specimen of choroideremia have indicated that this protein could perhaps be involved in pathological changes observed in this disease.

An extremely important new system for studying retinoblastoma cells in culture has been established by Dr. Athanassios Kyritsis. He has been able to establish human Y-79 retinoblastoma cells in attachment culture and to effect their differentiation into neuronal-like and glial-like elements. Of potential clinical importance, several natural agents have been identified that halt the growth of the tumor cells in culture.

A new enzyme activity has been identified in photoreceptor cell outer segments by Dr. Kapoor. This enzyme, called C-kinase, is activated by calcium and by phospholipids and uniquely phosphorylates several proteins of the photoreceptor. It may be that this enzyme is involved in the visual process since calcium is known to mimic many of the effects of light in the photoreceptor.

4. Office of the Chief: Dr. Andrew Mariani appears to have made an important observation in that he has identified a new, blue-sensitive, cone-bipolar system in the monkey retina. One of the great "mysteries" in retinal physiology has been the mechanism of color discrimination. The identification of this circuit should greatly aid the study of color vision.

Dr. Helen Hess has joined the LVR this year and has continued studies begun in the NEI Clinical Branch. Dr. Hess has worked with a strain of rats (RCS) which exhibit cataracts and retinal degeneration. She has found that nutritional and environmental factors play a role in cataract formation and is studying the effect of diet on the cataractogenic process.

5. Section on Experimental Immunology: Dr. Igal Gery continues to do outstanding work on the pathogenesis of immune-mediated eye diseases, attempting to understand them from both a basic science and a clinical viewpoint. In particular, the role of macrophages and of interleukin-1 in ocular inflammatory processes have been examined. Also, the experimental autoimmune uveitis (EAU) system has been further defined and has been shown to be a useful system in testing various drugs such as cyclosporine on immune-mediated eye diseases.

Dr. Paul Stein has joined Dr. Gery's group and is collaborating with Dr. Gery and Dr. Zigler on studying the roles of the S-antigen in uveitis. They have now shown that even proteolytic fragments of the antigen are capable of causing EAU in test animals, thus opening up a new area for investigating how this or other agents are involved in autoimmune disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00135-12 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry of Retina and Pigmented Epithelium in Health and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Helen H. Hess	M.D.	Medical Officer (Research)	LVR, NEI
Others:	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist Section on Lens and Cataract	LVR, NEI
	Toichiro Kuwabara	M.D.	Chief, Laboratory of Ophthalmic Pathology	LOP, NEI

COOPERATING UNITS *(if any)*
Veterinary Resources Branch, DRR, NIH (J. Knapka)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations are being conducted on the biochemistry of the sensory retina, pigmented epithelium, choroid, and biological fluids in normal and disease states, particularly in animal models of human retinal degenerations and in human retinal diseases. The effects of nutrition, genetic background, and environmental lighting (or darkness) on incidence and progress of chorioretinal degeneration and associated posterior subcapsular cataracts (PSC) are being studied in pink-eyed and black-eyed strains of retinal dystrophic Royal College of Surgeons rats. Factors involved in causing maturation of the cataracts are being elucidated. Nutritional and environmental factors favoring prevention of the cataracts are of special interest. Several diets have been found to prevent mature cataracts, and dark-rearing has been shown to prevent the microscopically-detectable PSC.

Project Description:

Additional Personnel Engaged on Project:

Theresa L. O'Keefe	B.A.	Biological Lab. Tech.	LVR, NEI
Irving V. Westney		Biological Aid	LVR, NEI

Objectives: To study the biochemistry of retinal photoreceptor, neuronal, glial, pigmented epithelial cells, and biological fluids in health and disease, and to explore possibilities for prevention or therapy of disease of the retina and/or pigmented epithelium-choroid when a biochemical abnormality has been identified. This exploration extends to possibilities of prevention or therapy of cataracts which often accompany retinal and/or pigmented epithelium-choroid diseases. Diseases in which the retinal pigmented epithelium is involved are of particular interest.

Methods Employed: Defined diets are prepared and fed to congenic unaffected and affected retinal dystrophic animals in controlled experiments. Clinical findings are recorded after indirect ophthalmoscopic and biomicroscopic examination and slitlamp examination. Postmortem examination of the eye by dissecting microscopy and of stained specimens by light microscopy is performed. At appropriate times, photography is used. Cells are enumerated on a hemocytometer grid. Analytical methods include flameless atomic absorption spectrophotometry, standard biochemical assays by spectrophotometry, fluorometry, and separation procedures.

Major Findings:

1. Dietary Prevention of Cataracts in the Pink-eyed Royal College of Surgeons (RCS) Rats: Last year we reported that a purified diet of the American Institute of Nutrition (AIN-76) prevented the mature cataracts of the RCS rat. Many fewer rats had a mature cataract in 1 year (only 2%), as compared with rats fed the NIH-07 natural ingredient diet (27%) or Charles River #3500 natural ingredient diet (29%). We have also noted that autoclaved #3500 permits fewer cataracts to occur, suggesting that volatile or fragile factors may be involved. We have repeated the study with AIN and confirmed the previous results. We are proceeding to test diets which are modifications of AIN. Another type of diet we have found prevents mature cataracts is NIH-42, which contains no fishmeal or other animal product. This diet was designed for toxicological studies. Both NIH-07 and #3500 diets contain fishmeal, and some types of fishmeal can lead to cataracts in trout. We have found that substitution of fishmeal for the casein in the AIN diet produces a diet that permits cataracts to occur at approximately the same incidence as with NIH-07 or #3500. On the other hand, increasing the calcium content of the AIN diet did not increase the incidence of mature cataracts. These ongoing studies illustrate the usefulness of the purified AIN diet in the attempt to identify factors involved in cataractogenesis and its prevention.

2. Studies of Posterior Subcapsular Cataracts (PSC) by Slitlamp and Dissecting and Slide Microscopy. All rats fed the different diets are examined either by slitlamp or dissecting or slide microscopy, or more than one of

these. Dr. Kuwabara's Laboratory has provided valuable assistance in processing eyes for slide microscopy to evaluate the effects of diets and of environmental lighting conditions on retinal and lens pathology.

3. Enumeration of Cells/sq. mm. in Vitreous Cortex of RCS Dystrophic and Control Rats at Different Ages. The cortex of the vitreous is dissected under microscopic view, stained with brilliant green, and placed on a hemocytometer grid. A 6 mm. sq. coverslip is applied, and the layer of cells (1 cell layer thick) is counted in a 1 sq mm area. In control rats, the cells are hyalocytes, or resident macrophages. In the dystrophic rats the number of cells/sq mm increases 5-8 fold at the time the slitlamp-detectable cataracts occur at about 50 postnatal days. The relationship of the macrophages to the retinal and lenticular pathology is being explored.

4. Effects of Dark-rearing on Cataract Incidence in Pink-eyed RCS Rats. Our previous studies showed that black-eyed RCS rats lacking the pink-eyed dilution (p) gene did not develop any mature cataracts in 1 year, regardless of type of diet. This suggested that dark-rearing might also have an important effect on incidence of cataracts. Theresa O'Keefe, a summer employee in 1983, became interested in this subject and as a senior at Mount Holyoke College, she conducted the following study. The college provided an animal laboratory with double doors that permitted total darkness, and all work was done in red light. NIH provided breeding RCS rats and NIH-07 rodent diet. Pregnant females were placed in darkness a week before parturition, and the progeny never saw light until they were examined at specified ages. The following procedures were done: (1) counting of macrophages in the vitreous cortex; (2) low power microscopic examination of dissected lenses floating in saline; and (3) fixation in 10% buffered formalin for histopathological study of lens and retina. The fixed eyes were sent to NEI for processing by Dr. Kuwabara's laboratory. Results were compared with similar studies done in the summers of 1983 and 1984, using congenic control pink- and black-eyed rats, and pink- and black-eyed dystrophic rats reared in cyclic light. The findings may be summarized as follows: (1) The total absence of light prevented the formation of microscopically-detectable PSC in pink-eyed RCS rats through the period of study--85 postnatal days. It seems likely that, if kept in the dark, these rats would never develop mature cataracts. (2) The total absence of light greatly reduced the rate of retinal degeneration in the pink-eyed RCS dystrophic rats, as noted by previous investigators. (3) The total absence of light also reduced the concentration of macrophages (cells/sq mm) in the vitreous cortex, or web, of the pink-eyed RCS dystrophic rats. (4) The macrophage concentration reached its most abrupt and highest peak in the pink-eyed RCS rats reared in cyclic light and its lowest level in pink-eyed RCS rats reared in darkness. This may indicate that the macrophage concentration is a measure of the amount of retinal degeneration that has been occurring at some previous time. (5) A sudden peak of macrophages at 50 postnatal days in the pink-eyed rat reared in cyclic light (5 to 8 fold that of control rats) may be related to the fact that all sectors of the retina degenerate at the same rate, thus producing a high concentration of products that may stimulate entrance of macrophages from the blood stream. (6) The lower concentrations and double peaks of macrophages in dark-reared pink-eyed RCS rats, as well as in black-eyed dystrophics may be related to the different rates of degeneration in

various sectors of the retina (10 days slower in the central, and 30 days slower in the superior peripheral area, as compared with the inferior peripheral area whose degeneration is unaffected by light or darkness). (7) The observation that dark-reared pink-eyed RCS rats have lower concentrations of macrophages than cyclic light reared black-eyed rats suggests that dark-rearing of black-eyed rats might reduce the rate of their retinal degeneration and their incidence of microscopically detectable cataracts.

5. Effects of Increased Intensity of Cyclic Light and of Constant Light on Cataract Incidence in Pink-eyed RCS Rats. In our customary dietary experiments, the illumination inside the cages has been 1-3 footcandles, to eliminate possible effects of light. Using the standard NIH-07 diet, we have also explored the effects of rearing rats in higher intensity light (cyclic or constant), beginning at different ages (1-31 postnatal days). Increased light levels have accentuated the occurrence of a lens defect visible by slitlamp as a "lake." Dr. Kuwabara has shown that this defect is one in which the more superficial lens fiber cells fail to elongate sufficiently to form the posterior suture. We are studying the ages of vulnerability, the factors affecting the total area of the defect, and factors involved in eventual elongation of the cells to produce closure of the suture, versus progression to form a mature cataract.

Significance to Biomedical Research and the Program of the Institute: Retinal deteriorations are the major cause of untreatable blindness in the United States and probably in the world. The retinal pigmented epithelium is becoming increasingly implicated as the primary site of many of these disease processes. Posterior subcapsular cataracts occur in various types of hereditary retinal degenerations, in humans and in RCS rats, as well as in older persons and in some persons treated with steroids. Nutritional and genetic factors are thought to play key roles in many human diseases and can often be studied in detail in animal models, giving an opportunity to develop ways to prevent or cure the diseases. Environmental light intensity as a factor in retinal and lenticular diseases deserves more attention. Information gained from these studies should contribute to our understanding of human disease and to initiating and conducting trials of possible therapeutic measures.

Proposed Course: The project will be continued with further emphasis on controlled trials of nutritional regimens and rigorous elucidation of nutritional variables in retinal dystrophic animals. Studies will continue on effects of darkness and light on retinal degeneration and cataracts in RCS rats. Collaborations will continue on biochemical factors in cataractogenesis.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Hess HH, Knapka JJ, Newsome DA, Westney IV, and Wartofsky L: Dietary prevention of cataracts in pink-eyed RCS rats. Lab Anim Sci (in press).

Hess HH, Knapka JJ, Newsome DA, and Westney IV: Dietary prevention of cataracts in the pink-eyed RCS rat model of hereditary retinal degeneration and cataract. Invest Ophthalmol Vis Sci 25(Suppl):114, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00137-02 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Organization of Neurons and Neurotransmitters in the Primate Retina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Andrew P. Mariani Ph.D. Staff Fellow LVR, NEI

COOPERATING UNITS *(if any)*
Laboratory of Preclinical Pharmacology, NIMH, ADAMHA (M. Hadjiconstantinou; N. Neff); Laboratory of Neurophysiology, NINCDS, NIH (A. Lasansky); Laboratory of Neurochemistry, NINCDS, NIH (R. Nelson)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The functional organization of the primate retina is investigated using techniques which identify single cells or single populations of neurons. With Golgi impregnation, electron microscopy (EM), Golgi-EM, formaldehyde-induced fluorescence (FIF) for catecholamines, and immunohistochemistry, the component elements of the neuronal circuitry are identified. By these studies of the structure, connections, synapses, and neurotransmitters of Macaca retina, the functional role of neurons in the processing of the visual image in the retina can be defined. Bipolar cells, a class of retinal interneuron with dendrites in the outer plexiform layer that contact photoreceptors, and axons terminating in the inner plexiform layer convey the receptive field center responses of ganglion cells. Now another morphological type of bipolar cell has been found in Golgi preparations of the rhesus monkey retina, and it displays selective and patterned distribution of its dendritic contacts with blue-sensitive cone pedicles in the outer plexiform layer. The discovery of the blue-cone bipolar cell and the subsequent identification of specific parts of the blue-sensitive retinal circuitry is a major advance in understanding the neuronal bases of color vision in the primate visual system.

PHS 6040 (Rev. 1/84)

GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To investigate the processing and modification of the visual image in the retinas of primates by identifying the component elements of the neuronal circuitry, their synaptic organization and neurotransmitters, and thus, their functional role in vision.

Methods Employed: Anatomical techniques that identify the structure, connections, synapses, neurotransmitters, and distribution of single identified neurons or homogeneous populations of neurons are employed. Specifically, these include but are not limited to electron microscopy (EM), Golgi-impregnation, Golgi-EM, reduced silver methods, formaldehyde-induced fluorescence for biogenic amines, and immunohistochemical labeling of neurotransmitters and neurotransmitter synthetic enzymes.

Major Findings: Bipolar cells are a class of retinal interneuron with dendrites in the outer plexiform layer that contact photoreceptors, and axons terminating in the inner plexiform layer where they are thought to convey the receptive field center responses of ganglion cells. In monkey retina, many of whose ganglion cells respond to stimuli appropriate for each of the three different cone mechanisms there are known to be five types of cone bipolar cells, flat and invaginating midget bipolar cells, diffuse flat and invaginating cone bipolar cells, and giant bistratified bipolar cells. Yet, in spite of the fact that many of the monkey's ganglion cells are specific for one of the different cone mechanisms, none of the bipolar cells are known to connect to specific morphologically identified counterparts of the different spectral types of cones as do bipolar cells of teleost retina. Now, however, another morphological type of bipolar cell has been found in Golgi preparations of the rhesus monkey retina, and it displays an apparently selective and patterned distribution of its dendritic contacts with cone pedicles in the outer plexiform layer. The intercone spacing of dendritic contacts and their distribution matches the intercone spacing and proportion of cones identified as blue-sensitive.

While there are many unusual electrophysiological and psychophysical features of the blue-sensitive system which distinguish it from the red- and green-sensitive cone pathways, the identification here of a specific part of the blue-sensitive retinal circuitry is a major step forward in attempts to unravel the neuronal bases for these differences.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the types of neurons, their interconnections, and the chemicals by which they communicate with one another is fundamental to the understanding of the normal function of the retina.

Proposed Course: These studies of the structure and function of the primate retina are continuing.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Mariani AP: A morphological basis for verticality detectors in the pigeon retina. Naturwissenschaften 70:368, 1983.

Mariani AP: The neuronal organization of the outer plexiform layer of the primate retina. Int Rev Cytol 86:285, 1984.

Mariani AP: Bipolar cells in monkey retina selective for the cones likely to be blue-sensitive. Nature 308:184, 1984.

Hadjiconstantinou M, Mariani AP, Panula P, Joh TH, and Neff NH: Immunohistochemical evidence for epinephrine-containing retinal amacrine cells. Neuroscience (in press).

Mariani AP, Kolb H, and Nelson R: Dopamine-containing amacrine cells of rhesus monkey retina parallel rods in spatial distribution. Brain Res (in press).

Mariani AP and Lasansky A: Chemical synapses between turtle photoreceptors. Brain Res (in press).

Mariani AP and Lasansky A: Ribbon synapses between turtle photoreceptors. Invest Ophthalmol Vis Sci 23(Suppl):202, 1984.

Hadjiconstantinou M, Mariani AP, Panula P, Joh TH, and Neff NH: Epinephrine-containing amacrine cells in a mammalian retina. Invest Ophthalmol Vis Sci 23(Suppl):283, 1984.

Nelson R, Kolb H, and Mariani AP: Dopamine-containing amacrine cells of rhesus monkey parallel rods in retinal distribution. Invest Ophthalmol Vis Sci 23(Suppl):87, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00066-06 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synaptic Chemistry of Retinal Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR, NEI
Others:	Judith D. Evans	Ph.D.	Staff Fellow	LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A combined biochemical and anatomical study is underway to identify neurotransmitters of retinal neurons and in neurons of visual pathways, to examine the development of neurotransmitter systems in retina, and to determine the role of chemical neurotransmitters in the processing of visual information. Two systems are being investigated: (1) the simple visual system of the horseshoe crab Limulus polyphemus, and (2) developing mammalian retinal neurons.

(1) Studies of neurotransmitters in the Limulus visual system revealed that centrifugal fibers which project from the brain to the retina and innervate photoreceptor cells synthesize, store, and release the biogenic amine octopamine and amine conjugates. Photoreceptors respond to octopamine by increasing intracellular cAMP, and octopamine causes the phosphorylation of at least two photoreceptor phosphoproteins. These studies show that the central nervous system can modulate the biochemistry of photoreceptor cells via centrifugal fibers. Some of the biochemical changes induced by centrifugal innervation may influence basic processes of photoreceptor cell function such as phototransduction, adaptation or membrane turnover.

(2) The development of synaptic function of mammalian retinal neurons is being studied in normal intact rat retinas as well as in monolayer cultures prepared from embryonic rat retinas. The histogenesis of different biochemical classes of amacrine cells is being examined as is the development of the ability of these neurons to synthesize and store neurotransmitter substances. The investigation of effects of depolarization on GABAergic function of retinal neurons in monolayer culture has continued using assays of glutamic acid decarboxylase (GAD) activity, localization of GAD-like immunoreactivity and GABA uptake autoradiography.

Project Description:

Additional Professional Personnel Engaged on Project: None

Objectives: Superimposed upon the anatomical wiring diagram of neurons in visual systems is a complex synaptic chemistry. Knowledge of the synaptic chemistry of these neurons is critical to our understanding of visual processing in normal retinas as well as the causes of blindness. Our aim is to: (1) identify neurotransmitter molecules in the visual system, (2) learn how the development of neurotransmitter systems is controlled in retinas, and (3) understand the function of individual neurotransmitters in the processing of visual information.

Methods Employed: Biochemical and anatomical techniques are being used in these studies, including high voltage paper electrophoresis, modern liquid chromatography, radioimmunoassays, light microscopic autoradiography, histofluorescence, and immunocytochemistry.

Major Findings:

1. Synaptic chemistry of retinal efferent fibers in the Limulus visual system. Octopamine stimulates the phosphorylation of two specific proteins associated with preparations enriched in ventral photoreceptor cells.

2. Synaptic chemistry of developing retinal neurons.

a. In the normal developing rat retina, glutamic acid decarboxylase (GAD) activity was first detected on embryonic day 15 and GABA synthesis and storage on embryonic day 16. This is very early in retinal development and correlates with the first appearance of choline acetyltransferase activity and ACh synthesis. The rate of GABA synthesis and GAD activity increased sharply at the end of the first postnatal week and correlates in time with the appearance of structural synapses in the inner plexiform layer of the retina.

b. A study of the histogenesis of cells that contain dopamine in the adult rat retina has been completed. Although biochemical parameters associated with dopaminergic function have not been detected in rat retinas until postnatal ages, many of the cells that contain dopamine in adult retinas leave the mitotic cycle between embryonic days 16 and 19. This raises the possibility that low and as yet undetectable levels of dopamine synthesis and release may be present in embryonic retinas, or that these cells leave the mitotic cycle long before they begin to express their final differentiated neurotransmitter chemistry.

c. The increase in rate of GABA synthesis and storage in rat retinal cells grown in monolayer culture induced by growing the cells in medium containing 50 mM K^+ can be blocked by Ca^{++} channel blockers and mimicked by veratridine. These results indicate that GABA synthesis in retinal neurons can be influenced by depolarization and that the effect of depolarization requires the influx of extracellular Ca^{++}.

d. Depolarization increases the number of cells in culture that take up GABA but does not increase the number of cells showing GAD-like immunoreactivity. Thus the relationship between GABA uptake and the expression of the enzyme for GABA synthesis is plastic. GABA uptake studies do not necessarily reveal the total population of neurons that have the potential for GABA synthesis.

Significance to Biomedical Research and the Program of the Institute: Synaptic chemistry of retinal efferent fibers in the Limulus visual system. Efferent innervation to retinas is common among many animals. The function of this efferent innervation is only beginning to be understood. There is now evidence from several different species that efferent innervation controls circadian changes in the sensitivity of the eye to light, photoreceptor cell sensitivity, and photoreceptor cell membrane turnover. Our experiments have suggested that efferent innervation can have profound effects on the biochemistry of photoreceptor cells. Therefore, the possibility should be explored that some retinal diseases may be caused by a defect in efferent innervation. Our identification of neurotransmitter in retinal efferent fibers of the Limulus visual system, a preparation that has been central to our understanding of basic visual processes, will allow for detailed investigations of the biochemical and electrophysiological mechanisms underlying efferent control of visual function.

Synaptic chemistry of rat retinal neurons developing in monolayer culture. Proper processing of visual information by the adult retina requires establishment of appropriate synaptic connections among retinal neurons during development. Studies of factors which influence the development of synaptic function of rat retinal neurons in culture are vital to our understanding of the effect of environmental and genetic influences on neurons in the intact developing retina.

Proposed Course:

1. Synaptic chemistry of retinal efferent fibers in the Limulus visual system.

a. A major effort will be directed toward characterizing the amine conjugates released from retinal efferent fibers and testing their activity on the function of Limulus eyes.

b. Studies of the effect of octopamine on the biochemistry and function of photoreceptor cells will be pursued.

2. Synaptic chemistry of rat retinal neurons developing in monolayer culture.

a. The effect of activity on the development of "GABAergic" function will be analyzed further. This study will be extended to include "cholinergic" function.

b. Immunocytochemical procedures will be used to identify different cell classes in the retinal cultures.

c. The histogenesis of GAD-containing cells will be pursued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation.

Publications:

Evans JA, Chamberlain SC, and Battelle B-A: Autoradiographic localization of newly synthesized octopamine to retinal efferents in the Limulus visual system. J Comp Neurol 219:369, 1983.

Battelle B-A and Evans JA: Octopamine release from centrifugal fibers of the Limulus peripheral visual system. J Neurochem 42:71, 1984.

Chamberlain SC, Battelle B-A, and Calman BC: Subcellular localization of neutral red staining in Limulus ventral photoreceptors. J Neurobiol 15:79, 1984.

Battelle B-A: Efferent innervation to Limulus eyes: organization, function and neurotransmitter chemistry. Trends in Neuroscience (in press).

Yeh HH, Battelle B-A, and Puro DG: Dopamine regulates synaptic transmission mediated by cholinergic neurons in rat retina. Neuroscience (in press).

Battelle B-A, Truckenmiller ME, and Pepper JW: Development of rat retinal neurons in monolayer culture: Effect of elevating the concentration of K^+ in the growth medium. Soc for Neurosci Abstr 9:1099, 1983.

Yeh HH, Battelle B-A, and Puro DG: Development of synaptic transmission in a model culture system: regulation by dopamine. Soc for Neurosci Abstr 9:611, 1983.

Chamberlain SC, Battelle B-A, and Wyse GA: Localization of serotonin-like immunoreactivity in Limulus protocerebrum. Soc for Neurosci Abstr 9:76, 1983.

Evans JA and Battelle B-A: Histogenesis of dopamine-containing neurons in rat retina. Invest Ophthalmol Vis Sci 25(Suppl):259, 1984.

Battelle B-A and Truckenmiller ME: Localization of glutamic acid decarboxylase like immunoreactivity and GABA uptake in cultures of cells dissociated from embryonic rat retinas. Invest Ophthalmol Vis Sci 25(Suppl):124, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00015-19 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Cell Biology of the Vertebrate Retina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul J. O'Brien	Ph.D.	Head, Section on Cell Biology	LVR, NEI
Others:	Robert St. Jules	Ph.D.	Staff Fellow	LVR, NEI
	Mary G. Wetzel	Ph.D.	Staff Fellow	LVR, NEI

COOPERATING UNITS *(if any)*
Laboratory of Cell Biology, National Institute of Mental Health (M. Zatz).

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on palmitic acid incorporation into bovine rhodopsin were extended to include the development of a cell-free soluble enzyme-substrate assay system and the demonstration that palmitoyl coenzyme A was the donor molecule in the transfer reaction.

Both in vivo and in vitro experiments with rat retinas resulted in the incorporation of labeled palmitic acid into rat rhodopsin. The palmitic acid appears to be associated with newly synthesized rhodopsin but its incorporation is a relatively late post-translational event that could be related to vectorial intracellular transport.

Palmitic acid also labels phospholipids, primarily phosphatidyl choline. Arachidonic acid labels phospholipids in the descending order of phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine.

Project Description:

Additional Personnel Engaged on Project:

Martin Zatz	M.D.	Medical Officer	LCB, NIMH

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. This project was designed to determine where and when sugars are added to rhodopsin and what role they and other molecular markers play in the transport and assembly of rhodopsin into disc membranes and in the process of shedding and phagocytosis of disc membranes. In addition, biochemical correlates to circadian photoreceptor shedding will be sought, particularly in relation to glycoprotein synthesis and function. Finally, specific carbohydrate receptors will be sought on pigment epithelial microvilli which could mediate interactions with photoreceptors.

Methods Employed: Ordinary biochemical techniques were used, such as intravitreal injection, incubation of retinas with radioactive precursors, cell fractionation, SDS gel electrophoresis, thin layer and column chromatography, scintillation counting, and autoradiography.

Major Findings: Bovine rhodopsin becomes labeled when retinas are incubated with radioactive palmitic acid. The label has been shown to be palmitic acid esterified directly to some portion of the rhodopsin molecule. Isolated rod outer segments also support this incorporation even when solubilized with detergents. The donor molecule appears to be palmitoyl coenzyme A. Rat retinas are also capable of carrying out this reaction when isolated and incubated with palmitate or when the palmitate is intravitreally injected in the intact rat. For the first few hours the label is associated with newly synthesized rhodopsin molecules but the incorporation is insensitive to inhibitors of protein synthesis or of Golgi enzymes.

Palmitic acid simultaneously labels rod outer segment phospholipids, primarily phosphatidyl choline. On the other hand arachidonic acid labels, in descending order, phosphatidyl choline, phosphatidyl inositol and phosphatidyl ethanolamine. The highest specific activity, by far, is in phosphatidyl inositol.

Significance to Biomedical Research and the Program of the Institute: Palmitic acid has been reported as a covalently bound component of several membrane proteins. There is evidence that the palmitate could be involved in membrane fusion, in intracellular sorting and transport mechanisms or in the specific functions of the proteins. Each of these processes has been studied in detail for rhodopsin, and thus radioactive palmitate could serve as a convenient marker for significant events in the life history of this unique retinal protein.

The labeling and turnover of rod outer segment phospholipids is related to the transfer of palmitate to rhodopsin since these lipids appear to serve as a reservoir for the continual replacement of rhodopsin-bound palmitate.

Furthermore, arachidonic acid, particularly as a component of phosphatidyl inositol, appears to produce a family of potentially important prostaglandins and leukotrienes in rod outer segments. Arachidonate could thus be involved in some short-lived events related to transduction.

Proposed Course: Attempts will be make to localize the subcellular site of palmitate incorporation as well as to identify that portion of the rhodopsin molecule to which the palmitate is attached. Additionally, a search will be made for biochemical processes, such as arachidonic acid release and metabolism, that occur in response to light onset, a signal that can trigger photoreceptor shedding under certain circumstances.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Phototransduction.

Publications:

O'Brien PJ and Zatz M: Acylation of bovine rhodopsin by [^{3}H] palmitic acid. J Biol Chem 259:5054, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00016-17 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biochemistry of Normal and Dystrophic Retinas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Paul J. O'Brien Ph.D. Head, Section on Cell Biology LVR, NEI

COOPERATING UNITS *(if any)*
School of Veterinary Medicine, University of Pennsylvania (G. Aguirre)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tritium labeled fucose and palmitic acid were injected into the vitreous chambers of the eyes of miniature poodles, both normals and animals affected with inherited progressive rod-cone degeneration. The fucose and palmitate were incorporated into an integral membrane protein having the properties of rhodopsin on column chromatography as well as SDS gel electrophoresis. Simultaneous injection of carbon labeled leucine resulted in doubly labeled rhodopsin. Neither rhodopsin synthesis nor its posttranslational modification by fucose or palmitate appeared to be altered in the affected poodles. Thus neither modification appears related to the reduced rate of disc membrane assembly in the dystrophic dog retina.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is to examine biochemical events unique to the retina, especially the synthesis of photoreceptor membrane components, in the retinas of vertebrates which can be affected by inherited retinal degenerations.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, column chromatography, and SDS gel electrophoresis.

Major Findings: Intravitreal injection of tritium labeled fucose or palmitic acid into the eyes of normal miniature poodles or dogs with inherited progressive rod-cone degeneration resulted in the incorporation of either label into rhodopsin which was purified by affinity chromatography on Con A-Sepharose and by SDS gel electrophoresis. Carbon labeled leucine, injected simultaneously, provided an internal reference. Neither polypeptide synthesis, measured by leucine labeling, nor the relative labeling of rhodopsin with fucose or palmitate showed any difference between the normal and affected dogs.

Significance to Biomedical Research and the Program of the Institute: Heretofore, fucose was thought to be a component of cone visual pigments but not of rhodopsin. In contrast to many warm-blooded and cold-blooded vertebrates, dogs appear to incorporate fucose into both rod and cone visual pigments. Similarly, only recently has it been shown that palmitic acid is a covalent component of certain membrane proteins, including rhodopsin. These studies show that neither the glycosylation of rhodopsin nor its posttranslational modification with palmitic acid are defective in the dystrophic poodle. Thus neither of these processes account for the reduced rate of disc membrane assembly in these dogs.

Proposed Course: Since both fucose and palmitic acid are novel components of rhodopsin, the labeled proteins will be hydrolyzed and the liberated labeled compounds identified by chromatography to establish their chemical structures. In addition, further studies will include comparative rates of retinal protein synthesis and rhodopsin synthesis in normal and affected dogs.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00125-04 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropharmacology of the Retina

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Donald G. Puro	M.D., Ph.D.	Medical Officer	LVR, NEI
Others:	Hermes H. Yeh	Ph.D.	Staff Fellow	LVR, NEI
	Masakatsu Fukuda	M.D., D.Sc.	Visiting Scientific	LVR, NEI
	Wu-Hong Tsai	Ph.D.	Visiting Fellow	LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Cell Biology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to help establish a scientific basis for the pharmacological manipulation of retinal function. Techniques in neuropharmacology, electrophysiology, and cell biology, including cell culture, are used to explore the actions and interactions of neurotransmitters, neuromodulators, hormones, and selected drugs on specific types of retinal neurons. Molecules have been identified which have long-term influences over important neuronal functions.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The long range goal of this project is to establish a scientific basis for pharmacological approach to the treatment of retinal dysfunction. The strategy for achieving this goal is to identify and investigate molecules that influence the function and growth of retinal neurons.

Specific, short-term objectives are to apply technical advances in electrophysiology, neuropharmacology, and cell biology, including cell culture, to: (1) explore the effects of neurotransmitters, neuromodulators, hormones, and selected drugs on the function of specific types of retinal neurons, (2) discover molecules which regulate the functional maturation of neurons of the retina, and (3) use a culture system to help identify and investigate possible etiologies of abnormal retinal development.

Methods Employed: We have developed a cell culture system to study acetylcholine-synthesizing neurons derived from the rat retina. The synaptic release of neurotransmitter from a single, visually identified, cholinergic neuron can be monitored continuously using this system. Putative neurotransmitters, neuromodulators, and drugs are applied near a retinal neuron by micoriontophoresis or pressure ejection from micropipets. The relatively new electrophysiological techniques of patch-clamping and whole cell patch recording permit the study of ionic activities of retinal neurons.

Major Findings:

1. Substances capable of having a long-term influence on information processing by retinal neurons have been identified and investigated. These substances include the putative retinal transmitters, dopamine, and vasointestinal peptide. Evidence points to a role for cyclic AMP in linking these extracellular signals with intracellular events which regulate important functions of retinal nerve cells.

2. Molecules that may serve as developmental signals for the functional maturation of the mammalian retina have been discovered. Findings indicate that insulin, glucocorticoid hormones, and dopamine interact to regulate the development of at least certain retinal neurons.

3. A culture system can be used to identify and investigate possible etiologies of abnormal retinal development. A culture system was applied to study the pathophysiology of fetal retinal neurons in order to perform assays that, at present, are virtually impossible to do in vivo. A major finding was that, during a critical period in gestation, the functional development of cholinergic retinal neurons derived from a fetal rat could be altered if the mother had received a steroid drug or had been stressed.

Significance to Biomedical Research and the Program of the Institute: Retinal dysfunction is a major cause of irreversible visual loss. Unfortunately, there are very few pharmacological agents that can help patients with retinal problems. This project is designed to help establish a scientific basic for a pharmacological approach to the problems of retinal dysfunction. We have identified natural and synthetic chemicals that can regulate important functions of retinal neurons. Knowledge of various regulatory molecules should lead eventually to the discovery of chemotherapeutic agents which could do such things as make injured retinal circuits work better or rescue neurons of the retina before they become irreversibly damaged.

Proposed Course: Priority will be placed on the study of the molecular pharmacology of mammalian retinal neurons. The actions of hormones, transmitters, neuromodulators, and drugs will be analyzed using recently developed biophysical methods.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmitters, and Adaptation

Publication:

Puro DG: Use of a culture system to identify possible causes of abnormal retinal development. Invest Ophthalmol Vis Sci 25:691, 1984.

Puro DG: Cholinergic transmission by embryonic retinal neurons in culture: Inhibition by dopamine. Dev Brain Res 9:79, 1983.

Puro DG and Yeh HH: Development of synaptic transmission by cholinergic neurons in culture. J Neurosci Res 10:241, 1983.

Yeh HH, Battelle B-A, and Puro DG: Maturation of neurotransmission at cholinergic synapses formed in culture by rat retinal neurons: Regulation by cyclic AMP. Dev Brain Res 10:63, 1983.

Puro DG and Agardh E: Insulin-mediated regulation of neuronal maturation. Science (in press).

Puro DG: Cholinergic systems. In Retinal Neurotransmitters and Modulators: Models for the Brain, Morgan WW, editor. Boca Raton, CRC Press (in press).

Yeh HH, Battelle B-A, and Puro DG: Dopamine regulates synaptic transmission mediated by cholinergic neurons of the rat retina. Neurosci (in press).

Agardh E, Yeh HH, Herrmann R, and Puro DG: GABA-mediated inhibition at cholinergic synapses formed by cultured retinal neurons. Brain Res (in press).

Fukuda M, Yeh HH, and Puro DG: The VIP system in retinal cell cultures: Histology and physiology. Invest Ophthalmol Vis Sci 25(Suppl):291, 1984.

Puro DG and Agardh E: Insulin-mediated regulation of cholinergic transmission by retinal neurons in culture. Invest Ophthalmol Vis Sci 25(Suppl):292, 1984.

Fukuda M, Yeh HH, and Puro DG: Expression of substance P- and VIP-like immunoreactivity by rat retinal neurons in cell culture. Soc Neurosci Abstr 9:802, 1983.

Yeh HH, Battelle B-A, and Puro DG: Development of synaptic transmission in a model culture system: Regulation by dopamine. Soc Neurosci Abstr 9:691, 1983.

Puro DG and Agardh E: Regulation of synaptic development: A role for insulin. Soc Neurosci Abstr 9:690, 1983.

Agardh E, Yeh HH, and Puro DG: GABA-mediated inhibition at synapses formed by cultured retinal neurons. Soc Neurosci Abstr 9:411, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00069-07 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Responses to Ocular Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LVR, NEI
Others:	Toichiro Kuwabara	M.D.	Head, Laboratory of Ophthalmic Pathology	LOP, NEI
	Manabu Mochizuki	M.D.	Visiting Associate	LVR, NEI
	Barbara Vistica	B.A.	Microbiologist	LVR, NEI
	Cathy McAllister	Ph.D.	Extramural Fellow	LVR, NEI

COOPERATING UNITS *(if any)*
Laboratory of Developmental and Molecular Immunity/National Institute of Child Health and Human Development (E. Hanna; M. Walker).

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.5	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at learning about the pathogenesis of immune-mediated eye diseases. The main effort has been focused on investigating an animal disease, experimental autoimmune uveitis (EAU), which is considered a model for certain eye diseases in man. EAU is induced by immunizing animals with a retinal protein, S-antigen, and may be adoptively transferred to naive animals by lymphocytes from immunized donors. Findings of note in the present studies include: 1. A correlation was found between the susceptibility to EAU and number of choroidal mast cells in rats of various inbred strains. This finding (a) supports the notion that mast cells play an important accessory role in the induction of EAU, and (b) indicates that genetically regulated susceptibility to an autoimmune disease may be mediated in part by the number of mast cells at the target organ site. 2. Lymphocytes of the "helper/inducer" subset were identified to be the ones responsible for the adoptive transfer of EAU; lymphocytes of the "suppressor/cytotoxic" subset had no such activity. This finding defines the immunopathogenic mechanism of EAU to be of the "delayed type hypersensitivity" rather than the "cytotoxic" type. 3. EAU development may be modulated by drugs. Adoptive transfer of EAU was enhanced when preceded by a single injection of cyclophosphamide. On the other hand, actively induced EAU was inhibited or delayed by various immunosuppressive drugs when given during the disease induction. Of the drugs tested, cyclosporine was found to be superior by its low toxicity and high efficacy. In addition, the immunosuppressive effect of cyclosporine was found to be augmented by the development of specific immunotolerance to S-antigen in rats treated with this drug. The data also show that EAU provides an experimental system for assessing different compounds for their effect on immune mediated eye disease.

Project Description:

Additional Personnel Engaged on Project:

David Chatham	Student	LVR, NEI

Objectives: This project is aimed at gaining new knowledge concerning the pathogenic processes of experimental autoimmune uveitis (EAU). This animal disease may serve in many aspects as a model for certain human ocular conditions. The specific studies were aimed at the following issues: (1) The role of mast cells in the pathogenesis of EAU. Previous studies (see NEI Annual Report for FY 1983) were extended to test the possible relationship between the number of choroidal mast cells and the susceptibility to EAU of rats of different genetically defined strains. (2) The adoptive transfer of EAU was further analyzed by determining the identity of the lymphocyte subset which transfers the disease and the presence on these cells of markers specific for "activated lymphocytes," in particular the peanut agglutinin (PNA) receptor. (3) The modulation of EAU by immunosuppressive drugs. The role of suppressor cells in down-regulating EAU development was examined by treating rats with cyclophosphamide, a drug known to selectively eliminate a subset of suppressor cells. On the other hand, the EAU system was used to compare the immunosuppressive efficacy of cyclosporine and other drugs which are routinely used for treatment of immune mediated eye diseases in man.

Methods Employed: Mast cells were counted in mounted choroidal membranes which were bleached by potassium permanganate and oxalic acid and stained with toluidine blue. EAU was induced in rats of various inbred strains by immunization with S-antigen emulsified in Freund's complete adjuvant, with or without the additional intraperitoneal injection of Bordetella pertussis bacteria, 10 billion per rat. Sensitized lymphocytes were obtained from draining lymph nodes of donors immunized with S-antigen. The lymphocytes were cultured for 3 days with S-antigen prior to being injected into naive rats. In some experiments, the lymphocytes were fractionated according to their surface antigens, W3/25 or OX-8, which are specific for the helper/inducer or the suppressor/cytotoxic subsets, respectively. The fractionation was carried out by using the "panning" technique with monoclonal antibodies to the aforementioned antigens. Peanut agglutinin (PNA) receptors were detected on the lymphocytes' surface by staining with a fluorescent PNA preparation, while W3/25 or OX-8 antigens were detected by incubating the cells with the monoclonal antibodies, followed by staining with fluorescent antibodies to mouse IgG. Stained cells were counted by a fluorescent activated cell sorter (ORTHO). EAU was evaluated by daily clinical observation and by histological examination. Immune responses to S-antigen were measured by skin tests (Arthus and delayed type), lymphocyte responses in vitro, and by the ELISA for circulating antibodies. Treatment with drugs was carried out by schedules indicated below. Cyclosporine was dissolved in olive oil and was injected intramuscularly, while other drugs were dissolved in phosphate buffered saline and injected intraperitoneally.

Major Findings:

1. A good correlation was found between the number of choroidal mast cells

and susceptibility to EAU among rats of different genetically-defined inbred strains. Rats of high responder strains (Lewis, CAR), with EAU developing in 100% of immunized animals, had mean values of 50.2 and 98.2 choroidal mast cells per square mm, respectively. In contrast, low responder BN rats (EAU develops in about 1/4 of animals) had less than 1 choroidal mast cell/square mm. Intermediate numbers of choroidal mast cells (9.5/square mm) were found in eyes of F1 hybrids of Lewis and BN rats ("LBNF"), which also exhibited an intermediate degree of susceptibility to induction of EAU. Intermediate levels of choroidal mast cells (11.2/square mm) were also found in eyes of LeR rats. These rats are derived from the Lewis strain and are poor responders to EAU (0/10 rats with disease) when immunized with the conventional emulsion of S-antigen and adjuvant, but are fully responsive when injected additionally with B. pertussis bacteria. The relationship between mast cells and EAU development was also indicated by the finding that the disease induction was accompanied by mast cell degranulation: a partial degranulation was found even before the disease onset while virtually complete degranulation was observed at the disease peak.

2. New data concerning the features of the lymphocytes which transfer EAU include: (a) The subset of helper/inducer lymphocytes, which carry the antigenic marker W3/25, was found to be responsible for the disease transfer. On the other hand, no such activity was found in the subset of suppressor/cytotoxic lymphocytes, which carry the OX-8 antigenic marker. (b) The increase in capacity to adoptively transfer EAU, which develops during incubation of presensitized lymphocytes with S-antigen or concanavalin A, was found to be accompanied by increase in expression of certain surface cell markers, namely, the receptor for PNA and antigens specific for the helper/inducer (W3/25) or suppressor/cytotoxic (OX-8) subsets.

3. Development of EAU could be modulated by drugs in various patterns: (a) adoptive transfer of the disease was enhanced by pretreating the recipient rats with a single dose of cyclophosphamide, 48 hrs prior to cell transfer. Pretreated rats showed earlier onset of disease, higher frequency of affected rats and more severe ocular changes. In addition, cyclophosphamide-treated recipients had higher immune responses to S-antigen. (b) Drugs administered during the period of EAU induction by active immunization with S-antigen produced different degrees of suppressive effects. Of the tested agents, cyclosporine was found the only one not to have toxic effects at immunosuppressive doses; other drugs caused mortality and/or weight loss at doses affecting the immune response. Moreover, cyclosporine was found superior to other drugs in its capacity to suppress the development of EAU: only cyclosporine, at 10 or 20 mg/kg/day, inhibited EAU development in all treated rats. EAU induction was also inhibited by cyclophosphamide, at 10 mg/kg/day, but only in of 3/6 treated rats, while other drugs (bredenine, colchicine or dexamethazone) only delayed the onset of disease. Cyclosporine at 40 mg/kg/day, was also the only drug capable of fully preventing EAU development when given daily from day 7 to 14 after immunization; cyclosphosphamide, at 20 mg/kg/day, prevented the disease in 2 of 6 treated rats whereas only delays in onset were found in rats treated by other drugs. Cyclosporine was also unique in its effect on the immune response to S-antigen in the immunized rats. This drug inhibited selectively the T cell specific immune response to S-antigen while having only a slight effect on the B cell-mediated antibody production, or on

the T lymphocyte response to polyclonal mitogens such as concanavalin A. On the other hand, other drugs, when affecting the immune response, were highly non-selective and similarly inhibited both T cell-mediated and antibody responses. Further examination of rats treated with cyclosporine showed that this drug's immunosuppressive effect could be attributed in part to the development of specific unresponsiveness (=immunotolerance) in the treated rats: rats immunized with S-antigen and treated with cyclosporine did not develop EAU when reimmunized with S-antigen 2 weeks after discontinuation of the drug treatment. However, such rats were fully susceptible to another disease, experimental allergic encephalomyelitis when immunized at that time with myelin basic protein.

Significance to Biomedical Research and the Program of the Institute: The finding concerning the correlation between number of choroidal mast cells and susceptibility to EAU provides new supportive evidence for the notion concerning the role of the mast cell in facilitating the development of EAU. Further support for this notion is provided by the finding that EAU development is accompanied by degranulation of mast cells in the eye. The findings also suggest that differences in susceptibility to autoimmune diseases among animals with different genetic makeup may derive in part from differences in the number of local mast cells. It is also of note that this study shows for the first time that striking differences exist between rats of different inbred strains in their number of local mast cells.

The characterization of the lymphocytes which transfer EAU provides information relevant to the understanding of these cells' immunopathogenic activity. Thus, the finding that these cells belong to the helper/inducer subset indicates that "delayed type hypersensitivity"-like reactions rather than "cytotoxic" reactions are those which bring about the ocular tissue damage. The data concerning the relationship between enhanced uveitogenicity and certain surface markers may provide information concerning the poorly understood "activation" process, as well as the mechanisms by which these cells invade the target organ and produce disease: it is conceivable that the PNA receptor plays a role in the "invasion" process.

Pretreatment with cyclophosphamide is known to selectively affect a population of suppressor cells, and thus the findings with recipient rats pretreated with this drug are interpreted to show that these cells may physiologically down-regulate the immunopathogenic mechanisms of uveitis.

The experiments concerning the effects of various immunosuppressive drugs on the development of EAU constitute to the best of our knowledge the first study in which drugs with different modes of action have been simultaneously compared for their effect on an immune-mediated ocular disease. Further, since EAU may be considered a model for certain ocular conditions in man, the findings of this study could be useful for future studies with human subjects. Of particular interest are the data demonstrating the unique features of cyclosporine, as compared to other immunosuppressive drugs. These include (a) the low toxicity, (b) high immunosuppressive efficacy, (c) selectivity toward T cell mediated processes, and (d) treatment with cyclosporine produces specific immunotolerance. The latter finding suggests that highly beneficial specific

immunotolerance may also develop in patients with autoimmune diseases which are treated with cyclosporine.

Proposed Course: The main issue to be dealt with concerns the immunopathogenic mechanisms of EAU. The system to be mostly used for these studies is the one of adoptive transfer of EAU. This system enables a fine analysis of the various processes which contribute to the pathogenesis of this immune-mediated ocular disease. More specifically, attempts will be made to further define the features of activated lymphocytes which transfer EAU, mainly with regard to their unique surface markers and the relationship between these features and the capacity of these cells to home to the target organ and to activate the pathogenic processes. In addition to the activation methods used in previous studies, the effect of microbial adjuvants will be examined, both in vivo and in vitro. A microbial product to be tested in particular is pertussigen, the active component of B. pertussis. In addition, the processes which take place in the recipient rat will be dissected out, mainly with regard to the "homing" pattern of the transferred lymphocytes and the recruitment processes which presumably take place in these animals.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Mochizuki M, Charley J, Kuwabara T, Nussenblatt RB, and Gery I: Involvement of the pineal gland in rats with experimental autoimmune uveitis. Invest Ophthal Vis Sci 24:1333, 1983.

Nussenblatt RB, Palestine AG, Rook AH, Scher I, Wacker WB, and Gery I: Cyclosporin A therapy of intraocular inflammatory disease. Lancet ii:235, 1983.

Nussenblatt RB, Palestine AF, Chan C-C, Leake WC, Rook AH, Scher I, and Gery I: Cyclosporine therapy in the treatment of uveitis. Transplant Proc 15 (Suppl 1):2364, 1983.

Mochizuki M, Nussenblatt RB, and Gery I: Effects of cyclosporine (CsA) on the efferent limb of the immune response. Transplant Proc 15 (Suppl 1):2364, 1983.

Gery I and Nussenblatt RB: Immunosuppressive drugs and therapy in ophthalmology. In Handbook of Experimental Pharmacology, Volume 69, Pharmacology of the Eye, Sears ML, editor. Berlin, Springer-Verlag, 1984, pp. 585-609.

Mochizuki M, Kuwabara T, Chan C-C, Nussenblatt RB, Metcalfe DD, and Gery I: An association between suscepticility to experimental autoimmune uveitis and choroidal mast cell numbers. J Immunol (in press).

Zigler JS Jr, Mochizuki M, Kuwabara T, and Gery I: Purification of retinal S-antigen to homogeneity by the criterion of gel electrophoresis silver stain. Invest Ophthalmol Vis Sci (in press).

Mochizuki M, Kuwabara T, McAllister C, Nussenblatt RB, and Gery I: Adoptive transfer of experimental autoimmune uveoretinitis in rats: immunopathogenic mechanisms and histological features. Invest Ophthalmol Vis Sci (in press).

Mochizuki M, Kuwabara T, Chan C-C, Nussenblatt RB, and Gery I: Choroidal mast cells and susceptibility to experimental autoimmune uveitis. In Proceedings of International Symposium on Uveitis, Saari KM, editor. Amsterdam, Elsevier Science Publishers B.V. (in press).

BenEzra D, Chan C-C, Gery I, Nussenblatt RB, and Palestine AG: Cross antigenicity between S-Ag and S-100 protein preparations. In Proceedings of International Symposium on Uveitis, Saari KM, editor. Amsterdam, Elsevier Science Publishers B.V. (in press).

McAllister C, Mochizuki M, Hanna E, Walker M, Kuwabara T, and Gery I: Adoptive transfer of experimental autoimmune uveitis: Characterization of the cells and processes involved. Invest Ophthalmol Vis Sci 25 (Suppl):29, 1984.

Gery I, Mochizuki M, Kuwabara T, and Zigler JS Jr: Purification of S-antigen by high performance liquid chromatography (HPLC). Invest Ophthalmol Vis Sci 25 (Suppl):39, 1984.

Chan C-C, Mochizuki M, Nussenblatt RB, Palestine AG, Gery I, and BenEzra D: T lymphocyte subsets in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 25 (Suppl):39, 1984.

Mochizuki M, Nussenblatt RB, Kuwabara T, and Gery I: Effects of Cyclosporine and other immunosuppressive drugs on experimental autoimmune uveoretinitis in rats. Invest Ophthalmol Vis Sci (in press)

McAllister C, Mochizuki M, Kuwabara T, Hanna E, Walker M, and Gery I: Adoptive transfer of experimental autoimmune uveitis (EAU). Fed Proc 43:1994, 1984.

Mochizuki M, Kuwabara T, Chan CC, Nussenblatt RB, Metcalfe DD, and Gery I: Choroidal mast cells and susceptibility to experimental autoimmune uveitis (EAU). Fed Proc 43:1994, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00023-06 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Macrophage Interactions with Other Cells and Their Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Igal Gery	Ph.D	Head, Section on Experimental Immunology	LVR, NEI
Others:	Jose Luis Lepe-Zuniga	M.D.	Visiting Fellow	LVR, NEI
	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LVR, NEI
	Barbara Detrick-Hooks	Ph.D.	Expert	LVR, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project encompasses studies aimed at analyzing the participation of macrophages and their products in processes which affect the eye or other tissues. The experiments reported here were a continuation of the ongoing study concerning interleukin 1 (IL-1), a macrophage product which plays a major role in mediating certain immune responses, inflammatory processes, and wound healing. Noteworthy findings: (1) Previous studies have shown that macrophages may retain high levels of intracellular IL-1 and that this pool of IL-1 differs by certain aspects from the extracellular pool, which is released into the medium. The present experiments have revealed that the two pools of IL-1 also differ markedly in their molecular charge, as demonstrated by their isoelectrofocusing profile. A major portion (about 60%) of the the extracellular pool has an isoelectric point (pI) of 6.7, while smaller portions are characterized by pIs of 5.5 and 6.1. On the other hand, about 90% of the intracellular IL-1 pool was found to have a pI of 5.5 and only minor portions were found with pI of 6.7 or 6.1. (2) We have previously proposed that intracellular IL-1 is mainly involved in facilitating the immune response while the extracellular pool's function is to mediate the body's reaction to injury or inflammation. This hypothesis was examined by measuring the levels of extracellular and intracellular IL-1 activities in macrophage cultures stimulated with lipopolysaccharide (endotoxin) or one of its components, lipid A, which was treated to lose its capacity to cause fever but to retain its immune enhancing capacity. The detoxified lipid A was found to selectively increase the intracellular IL-1 activity while having less effect on the extracellular pool. On the other hand, untreated lipid A, or the whole lipopolysaccharide molecule, stimulated similar levels of both intracellular and extracellular IL-1 activities. The data are interpreted to support the aforementioned hypothesis by showing a correlation between the capacity to increase the intracellular IL-1 and immune enhancement.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: The present phase of this project has extended studies concerning interleukin 1 (IL-1), a macrophage product which mediates a variety of processes related to immunity, inflammation, and wound healing. The present experiments have dealt with two issues: (1) the biophysical properties of the intra- and extracellular pools of IL-1 were further compared, in order to better analyze the relationship between these two pools. (2) The effect of detoxified lipid A of LPS on the production and release of IL-1 was monitored. It is assumed that the toxicity of native lipid A or the whole LPS molecule is due to their capacity to stimulate the release of endogenous pyrogen, a macrophage product which closely resembles IL-1 and may be identical to it. On the other hand, we have hypothesized that the immune enhancing effect of LPS and its components is related to the increase in the intracellular level of IL-1 in stimulated macrophages. The availability of detoxified lipid A has provided one approach to test the hypothesis, by determining whether this component of LPS selectively stimulates intracellular IL-1 activity.

Methods Employed: The macrophage cultures used in this study consisted of monolayers of human monocytes or mouse peritoneal macrophages, prepared by routine methods. The stimuli included lipopolysaccharide (LPS) or its component lipid A in its native form or after being "detoxified" by removal of one phosphate group. Both forms of lipid A were provided by Ribi Immunochem, Hamilton, MT. Supernatants of the stimulated cultures were used as the extracellular pool of IL-1, while the intracellular pool consisted of the cell lysates. Fractionation of the two IL-1 pools by isoelectrofocusing was carried out by a conventional method using an LKB Multiphor unit. Levels of IL-1 were determined by the conventional assay, i.e., stimulation of murine thymocyte cultures.

Major Findings:

1. The intracellular pool of IL-1 from human monocytes was found to differ markedly from the extracellular pool in its isoelectrofocusing profile. As reported by other investigators, the extracellular pool was found to be composed of three molecular species, with isoelectric points (pI) of 5.5, 6.1 and 6.7. A major portion of the activity, approximately 60%, localized at the pI 6.7 peak, about 30% localized at the pI 6.1 peak, while 10% or less of the IL-1 activity was found at the pI 5.5 peak. On the other hand, approximately 90% of the intracellular activity was confined to the peak at pI 5.5 and the remaining activity was found to be distributed between the peaks at pI 6.1 and 6.7.

2. A preparation of detoxified (non-pyrogenic) lipid A was found to differ from the native lipid A, or from the original LPS molecule, by producing more intracellular than extracellular IL-1 activity. This pattern of IL-1 production and release was observed when the different preparations were added to cultures of either human monocytes or murine macrophages.

Significance to Biomedical Research and the Program of the Institute: The

finding of marked differences between the isoelectrofocusing profiles of the intra- and extracellular pools of IL-1 is a new indication that the two pools differ in their biophysical properties. Our previous studies (Annual Report 1983) showed that the two pools of IL-1 also differ in their molecular size profile and kinetics of production. Put together, our findings may thus support the notion that the two pools of IL-1 also differ in their biological function: we have proposed that the intracellular pool is mainly involved in stimulation of immune responses, while the extracellular pool is used to mediate the body's response to tissue injury and inflammation.

The finding that detoxified lipid A induces more intracellular than extracellular IL-1 is in line with the aforementioned notion concerning the different functions of intra- and extracellular IL-1 pools. More specifically, this finding supports the assumption that the toxicity of lipid A and LPS is related to the release of the IL-1-like monokine, endogenous pyrogen. Further, since the detoxified lipid A was reported to retain its adjuvant capacity, the reported finding supports the notion that intracellular IL-1 (and not the extracellular activity) has a role in facilitating immune responses. The data are also of interest for researchers dealing with induction of uveitis. Because a form of anterior uveitis may be produced in animals by LPS, the use of lipid A or its detoxified form could be useful in further analysis of the pathogenic process.

Proposed Course: The activities of detoxified lipid A will be further analysed with regard to its capability to induce IL-1 and to act as an adjuvant in autoimmune responses, both in vivo and in vitro. The selective effect of detoxified lipid A in increasing intracellular IL-1 will be employed to examine the relationship between IL-1 and the other macrophage products which induce fever or increase the synthesis of acute phase proteins.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Zigler JS Jr, Gery I, and Kinoshita JH: Effects of lipid peroxidation products on the rat lens in organ culture: a possible mechanism of cataract initiation in retinal degenerative disease. Arch Biochem Biophys 225:149, 1983.

Gery I, Benjamin WR, Jones S, and Nussenblatt RB: Cyclosporine (CsA) selectively inhibits certain mitotic responses of thymocytes. Transpl Proc 15 (Suppl 1):2311, 1983.

Mochizuki M, Zigler JS Jr, Russell P, and Gery I: Serum proteins neutralize the toxic effect of lysophosphatidyl choline. Curr Eye Res 2:621, 1982/1983.

Gery I and Lepe-Zuniga JL: Interleukin 1: uniqueness of its production and spectrum of activities. In Lymphokines, Pick E, editor. New York, Academic Press, 1984, Vol. 9, pp. 109-125.

Schmidt JA, Oliver CN, Lepe-Zuniga JL, Green I, and Gery I: Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1: A potential role for IL-1 in the pathogenesis of silicosis. J Clin Invest 73:1462, 1984.

Gery I and Schmidt JA: Human interleukin 1. In Methods in Enzymology, Volume on Immunochemical Techniques, diSabato G, Langone JJ, and Van Vunakis H, editors. New York, Academic Press (in press).

Lepe-Zuniga JL and Gery I: Production of intra and extracellular interleukin 1 (IL-1) by human monocytes. Clin Immunol Immunopathol 31:222, 1984.

Mochizuki M, Zigler JS Jr, Russell P, and Gery I: Cytostatic and cytolytic activities of macrophages: regulation by prostaglandins. Cell Immunol 83:34, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 000190-01 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification and Characterization of S-antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul Stein	Ph.D.	Expert	LVR, NEI
Others:	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LVR, NEI
	Igal Gery	Ph.D.	Head, Section on Experimental Immunology	LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Immunology

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

S-antigen, a protein located in retinas of all vertebrates, is of importance for its capacity to induce a disease, experimental autoimmune uveitis (EAU) in animals immunized with microgram amounts of this antigen. Further, accumulating data suggest that S-antigen may be involved in certain pathogenic autoimmune processes in the human eye. The purpose of this project is to purify and characterize this antigen, in order to better understand its immunopathogenic effect. Using the technology of "high performance liquid chromatography" (HPLC), a procedure was devised for purification of S-antigen to homogeneity. Purified preparations will be useful for further studies concerning the identity and biological function of S-antigen. Other experiments showed that S-antigen is resistant to boiling and detergent treatment, which increase the digestability of the molecule by enzymes. Digestion with a bacterial proteolytic enzyme was found to degrade S-antigen to fragments of small molecular sizes. Rats immunized with a pool of these fragments developed EAU, thus showing that these components retain the capacity to cause EAU.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: Experimental autoimmune uveitis (EAU) is an inflammatory disease which is induced in various animals by immunization with the retinal S-antigen. The objectives of this study are: (1) To develop a method for the purification of S-antigen to homogeneity; (2) To collect data concerning the antigenic and biophysical properties of S-antigen; and (3) To digest the S-antigen molecule, in order to identify its uveitogenic component(s).

Methods Employed: The S-antigen was isolated from bovine retinas by conventional liquid chromatography procedures followed by high performance liquid chromatography (HPLC) on both anion exchange and gel exclusion columns. Immunochemical techniques using antisera to S-antigen and gel electrophoresis with silver staining were used for identification of S-antigen and as criteria of purity. Larger quantities of partially purified S-antigen were obtained from bovine or guinea pig retinas by use of only the conventional procedures utilizing gel filtration and ion-exchange liquid chromatography methods. Enzymatic digestion of S-antigen was carried out by incubating native S-antigen or heated and SDS-treated S-antigen with varying concentrations of S. aureus V8 protease. The enzymatic effects were analyzed by polyacrylamide gel electrophoresis (PAGE), followed by silver staining or by transblotting the bands onto nitrocellulose membranes ("Western blotting") and probing with antibodies to S-antigen. The antibodies used were polyclonal (rat or rabbit) or monoclonal (mouse). Uveitogenicity of S-antigen preparations was determined by their capability to induce EAU in Lewis inbred rats following immunization in complete Freund's adjuvant emulsion. Antigenicity of the S-antigen preparations was assessed by the ability (1) to react in immunodiffusion with the specific antibodies, (2) to provoke skin responses (Arthus or delayed type) in rats immunized against S-antigen, or (3) to stimulate increased thymidine incorporation in cultures of lymphocytes from rats immunized against S-antigen.

Major Findings:

1. Conventionally purified S-antigen contains about 80% S-antigen and a large number of minor contaminants which become readily apparent by silver staining of electrophoretic gels. Further purification of such material by HPLC anion exchange chromatography followed by gel exclusion chromatography on an HPLC column produced a preparation of S-antigen which was homogeneous by the criteria of electrophoresis with silver staining. The material prepared by this new technique remained fully reactive with anti-S antigen serum and was more potent than conventional S-antigen preparations in the induction of EAU in rats.

2. Heating (100°C, 5 min) did not destroy but partially reduced the uveitogenic capacity of either bovine or guinea pig S-antigen. A similar effect was observed when heating was performed in the presence of SDS. Immunodiffusion analysis of heated S-antigen revealed that partial denaturation occurred, as indicated by "spur" formation when tested beside the native preparation. Heated S-antigen was also found to resemble the native antigen in its capacity to stimulate lymphocytes from rats immunized with either form of S-antigen.

3. Digestion of native bovine S-antigen (not exposed to SDS detergent and heating) resulted in the disappearance of the S-antigen (50Kd) protein band and subsequent appearance of a smaller molecular weight fragment (approximately 45 Kd). This "breakdown" fragment was found to induce EAU in Lewis rats and also formed a precipitin band against anti-rabbit S-antigen identical to that with native S-antigen.

4. Protease digestion of heated and SDS-treated S-antigen produced several fragments which could be detected by PAGE. After Western blotting, some fragments were found to bind specific antibodies to S-antigen, suggesting that the same antigenic site is present in these fragments. A pool of these fragments, obtained from the PAGE gel, was found to induce EAU in Lewis rats.

Significance to Biomedical Research and the Program of the Institute: (1) The HPLC technology was found to provide a method by which S-antigen can be purified to homogeneity. Such purified preparations are essential for further studies concerning the identity and biological function of this molecule. (2) Data on the heating effect show that the S-antigen molecule is much more heat resistant than previously reported. The heat resistance may be useful for further cleavage studies in which detergent (SDS) treatment is employed. (3) The preliminary results concerning the enzymatic cleavage of S-antigen demonstrate that this approach may be used to obtain fragments of the antigen which carry the uveitogenic capacity of the intact molecule. These findings should be useful, therefore, in elucidating the molecular mechanisms whereby S-antigen induces EAU, which is considered an animal model for certain inflammatory conditions in the human eye.

Proposed Course: The procedures developed so far will be used to purify and characterize cleavage fragments of S-antigen which carry uveitogenic capacity. The main effort will be focused on the biochemical and biophysical features of the fragments and on their immunogenic and antigenic properties.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Zigler JS Jr, Mochizuki M, Kuwabara T, and Gery I: Purification of retinal S-antigen to homogeneity by the criterion of gel electrophoresis silver staining. Invest Ophthalmol Vis Sci (in press).

Gery I, Mochizuki M, Kuwabara T, and Zigler JS Jr: Purification of S-antigen by high performance liquid chromatography (HPLC). Invest Ophthalmol Vis Sci 25(Suppl):29, 1984.

Stein PC: Experimental autoimmune uveitis induced by protease digests of bovine S-antigen in Lewis rats. Invest Ophthalmol Vis Sci 25(Suppl):30, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00139-02 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hydrogen Peroxide Activation in Damage to the Ocular Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard Steven Bodaness M.D., Ph.D. Senior Staff Fellow LVR, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Lens and Cataract

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aqueous humor of the human eye is known to contain hydrogen peroxide at concentrations higher than that present in most other tissues. Hydrogen peroxide is relatively non-toxic in micromolar concentrations, but may serve as a stable precursor for more potent toxic oxidant species. Present investigations center on the role of hydrogen peroxide as a toxic oxidant precursor, mechanisms of hydrogen peroxide activation, identification of hydrogen peroxide-derived oxidants, and the role of hydrogen peroxide activation in diseases of the lens and other ocular tissues.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

J. Samuel Zigler, Jr. Ph.D. Research Biologist LVR, NEI

Objectives: The purpose of this work is twofold: (1) to explore in a systematic manner the major aspects of hydrogen peroxide and lipid hydroperoxide activation in relation to diseases of the lens and other ocular tissues, and (2) to explore the non-photodynamic effects of photosensitizing molecules (including photosensitizing drugs) on enzymes which are involved in the metabolism of hydrogen peroxide. The human aqueous humor is known to contain hydrogen peroxide at higher concentrations than that present in almost all other tissues. Therefore, the activation of this molecule to markedly more potent oxidative toxic species may function etiologically and mechanistically in diseases of the lens and other ocular tissues. The objective of these studies is to understand how peroxide activation leads to tissue damage.

Methods Employed: The heme-undecapeptide from cytochrome c was utilized to generate reactive oxygen species from hydrogen peroxide. Oxidative activity has been evaluated utilizing absorption spectroscopy, fluorescence emission spectroscopy, and sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Major Findings:

1. The system utilized was that previously used for the study of reduced pyridine nucleotide (NADPH) oxidation. It consists in the activation of hydrogen peroxide by the heme-undecapeptide from cytochrome c, and was subsequently applied to the oxidation of lens crystallins. With this system for the generation of potent reactive oxidant species from hydrogen peroxide, it was demonstrated that of the three major classes of lens crystallins only γ-crystallin is crosslinked by the H_2O_2-heme-undecapeptide oxidative system, whereas α and β crystallins are not.

2. Heme-undecapeptide plus hydrogen peroxide generates dityrosine from free tyrosine. Concomitant with crosslinking, when γ-crystallin is exposed to the generated oxidant, it develops a new fluorophor with the characteristics of dityrosine. The findings with bovine and human crystallins are identical in this regard.

3. The crosslinking was markedly inhibited by 1 mM concentrations of tryptophan, tyrosine, and cysteine.

4. Exposure of tryptophan to the heme undecapeptide-H_2O_2 oxidative system results in a decrease in tryptophan fluorescence, but does not result in the formation of a new fluorophor.

5. The intrinsic fluorescence of all three major classes of lens crystallins, α, β, and γ, is due primarily to tryptophan. The intrinsic fluorescence of each of α, β, and γ-crystallin is decreased by the oxidant from heme peptide-H_2O_2.

6. Therefore, it can be seen that tryptophan oxidation occurs in all crystallins, but crosslinking occurs only in γ-crystallin and is associated with oxidation of tyrosine.

Significance to Biomedical Research and the Program of the Institute: These studies are of major significance to the program of the National Eye Institute because of their potential relevance to the etiology of various forms of lenticular cataract. In addition to senile cataract, lenticular opacification is a well known component of a diversity of diseases. The experiments outlined herein may point the way to the development of therapeutic modalities which can act to prevent or impede cataract formation.

Proposed Course: The following studies are in progress or proposed for FY 1985:

1. The precise identification of the specific oxidant generated by hydrogen peroxide and heme-peptide.
2. An analysis and identification of compounds which may serve as drugs to either prevent the generation of toxic species from hydrogen peroxide or to scavenge these species once they are formed.
3. An analysis of the ability of the heme peptide-hydrogen peroxide oxidant to damage other ocular tissues such as the retina (which is rich in unsaturated lipids) that can convert to lipid hydroperoxide.
4. An analysis of the ability of heme-peptide to react with peroxides of unsaturated lipids.

NEI Research Program: Cataract--Cataract Induced by Environmental and Toxic Effects

Publications:

Bodaness RS, LeClair M, and Zigler JS Jr: An analysis of the H_2O_2-mediated crosslinking of lens crystallin catalyzed by the heme-undecapeptide from cytochrome c. Arch Biochem Biophys 231:461, 1984.

Bodaness RS: The non-photosensitized potentiation by the photosensitizer hematoporphyrin of the horseradish peroxidase-catalyzed H_2O_2-mediated oxidation of NADPH to $NADP^+$. Biochem Biophys Res Commun 118:191, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 000189-01 LVR

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Role of Protein Kinases in Lens Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Donita L. Garland Ph.D. Expert LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Vision Research

SECTION

Lens and Cataract Section

INSTITUTE AND LOCATION

NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The role of protein kinases in the lens has been addressed by studying the endogenous proteins that serve as substrate for the endogenous protein kinases. Two intrinsic membrane proteins MP26 and MP19 are phosphorylated by lens cAMP-dependent protein kinase. In the cortex, cytoskeletal proteins, including the intermediate filament protein, vimentin, are phosphorylated. Oxidative changes of proteins are thought to occur with aging and to contribute to the development of light scattering in the lens. In certain types of cataracts, an increase in oxidative modifications was demonstrated. Studies are in progress to determine the nature of the modifications and the mechanisms leading to the changes.

PHS 6040 (Rev 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: (1) To determine whether protein kinases and phosphoprotein phosphatases have a role in the regulation of lens metabolism. (2) To determine whether changes in the phosphorylation or dephosphorylation of specific lens proteins could lead to formation of light scattering in lens. (3) To understand mechanisms of oxidative modification of proteins observed in aging and in certain types of cataracts.

Methods Employed: Bovine lenses, aged 4 months to 5 years and normal and cataractous human lenses were used for these studies. Enzyme assays involved the use of radiolabeled compounds. Identification of labeled proteins involved the use of standard electrophoretic, immunologic, and autoradiographic techniques.

Major Findings:

1. Two intrinsic proteins of purified calf lens fiber cell membranes can be phosphorylated by cAMP dependent protein kinases. The proteins MP26 and MP19 are phosphorylated by cAMP dependent protein kinases from bovine lens cortex and rabbit muscle.

2. Serine is the only phospho-amino acid detected in MP26 and MP19 when phosphorylated by either the lens or rabbit muscle protein kinase.

3. Trypsin, chymotrypsin and Staphylococcus aureus V8 protease treatment of phosphorylated membranes removes the phosphorylation site(s) from MP26 and MP19. For MP26 this indicates the phosphorylation site(s) is within a 4-5 kDa segment from the carboxy terminus.

4. The identity of the 19 kDa phosphorylated protein is not known. Antibodies directed against α, β and γ crystallins (a gift from Dr. Zigler) do not crossreact with this protein suggesting it is not a crystallin.

5. Incubation of the buffer insoluble fraction of calf lenses with cAMP or in the absence of cAMP leads to the phosphorylation of several proteins. In the cortex, proteins with molecular weights of 43, 57 and 95,000 are phosphorylated in the absence of added cAMP. In the nucleus there is little ^{32}P incorporated into the high molecular weight proteins. When cAMP is added, ^{32}P is incorporated into the 19 kDa protein in both the cortex and nucleus. In the nucleus there is little phosphorylation of MP26.

6. Several observations suggest the 56 kDa phospho-protein in the cortex is the intermediate filament protein, vimentin. The protein and ^{32}P incorporation are diminished in nuclear preparations relative to the cortex samples. The addition of partially purified vimentin increases the ^{32}P at this molecular weight. Using immunoblot techniques, anti-vimentin antibodies label a protein that comigrates with the 56 kDa phospho-protein in the cortex, but the labeling

by antibody is diminished in the nuclear preparations.

The carbonyl specific reagent 2,4-dinitrophenylhydrazine (DNPH) was used to assay for "oxidized" protein in bovine lenses of different ages, human lenses aged 47-82, and various types of cataractous lenses.

7. There is no significant increase in the carbonyl content of bovine lens from 4 months to 5-6 years of age. Two non-enzymic oxidation systems were used to determine the susceptibility of crystallins to this type of modification. The maximum level of modification of the crystallins was about 6%. These results suggest crystallins are relatively resistant to this type of modification or once modified, react further.

8. The carbonyl content of the TCA precipitable protein of normal human lenses is low and does not appear to increase with increasing age. The carbonyl content of the nuclear fractions is not significantly higher than that in the cortical fractions.

9. The carbonyl content is increased in some cataractous lenses, particularly brunescent cataracts. About 30% of the dinitrophenyl hydrazine is associated with guanidine-HCl-insoluble material. The hydrazone is solubilized by pepsin treatment indicating the carbonyl is associated with large protein aggregates.

10. The absorption maxima of the 2,4-dinitrophenylhydrazones formed in normal human lenses ranges from 360-367 nm. In cataracts the guanidine-HCl soluble hydrazones have absorption maxima of 359-365 nm. These are all in the range of aliphatic ketones and aldehydes. The absorption maxima of the guanidine-HCl-insoluble hydrazones are 370-375 nm. These fall in the range of olefinic ketones and aldehydes. More evidence is required to determine if the parent compounds of the carbonyls are protein, carbohydrate, or membrane.

Significance to Biomedical Research and the Program of the Institute: The accumulation of oxidatively modified protein is thought to occur with aging, and the oxidation of lens proteins is thought to be associated with cataract formation. These studies demonstrate an increase in oxidative modification in certain cataracts. Studies are now directed at characterizing the changes which occur and determining the mechanisms involved.

The studies on the phosphorylation-dephosphorylation of lens proteins are one approach to understanding the mechanisms which alter protein-protein interactions in the lens. The alterations which occur with age are thought to be a major cause of light scattering in the lens. These studies should also provide further understanding of the regulation of lens metabolism.

Proposed Course: Further research will include:

1. Studies on the regulation of the phosphorylation of the two intrinsic membrane proteins, MP26 and MP19. Preliminary studies indicate calcium and ypossibly calodulin are involved in the regulation.
2. Further characterization of the 19 kDa membrane phospho-protein.
3. Purification and characterization of protein kinases as a function of

age of lenses. The lens proteins which serve as substrates for the endogenous protein kinases will be characterized.

4. Studies will be directed towards identifying the nature of the carbonyls found in brunesent cataracts.

5. Examining individual enzymes as a function of age to determine if oxidative modifications occur.

NEI Research Program: Cataract--The Normal Lens

Publications:

Garland D and Nimmo H: A comparison of the phosphorylated and unphosphorylated forms of isocitrate dehydrogenase from Escherichia Coli ML308. FEBS Lett 165:259, 1984.

Russell P, Schwab SJ, and Garland D: The characteristics of monkey lens glyceraldehyde-3-phosphate dehydrogenase. Exp Eye Res (in press).

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Garland D, Clark JI, and Benedek G: Calf lens protein kinases phosphorylate endogenous proteins. Biochemistry 22:16A, 1983.

Garland D: Phosphorylation of lens fiber cell membrane proteins. Invest Opthalmol Vis Sci 25(Suppl):141, 1984.

Garland D, Stadtman ER, and Kinoshita, J: Oxidative modification of human lens. Fed Proc Abstr 43:2021, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00003-12 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cataracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter F. Kador Ph.D. Research Chemist LVR, NEI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Lens and Cataract

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	3.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Current investigations are being conducted on the events leading to the formation of several types of cataracts. Diabetic and galactosemic cataract formation, inhibited by the enzyme aldose reductase, are being studied as well as methods for controlling the onset of these cataracts through the regulation of aldose reductase. The relationship between aldose reductase and other ocular diabetic complications such as retinopathy, corneal epitheliopathy, and basement membrane thickening is also being investigated. Methods for delaying the onset of these complications through the pharmacological control of aldose reductase are also being developed.

The potential role of the enzyme pyrroline-5-reductase as well as the pyrroline-5-carboxylate (P-5-C) cycle through which ornithine and glutamine are converted to proline are also being investigated. Pyrroline-5-reductase has been proposed to regulate cellular redox potentials and increase ATP levels through stimulation of the pentose shunt. Cataracts are associated with patients with gyrate atrophy of the choroid and retina, a disease in which high ornithine levels result from the absence of the key enzyme ornithine amino transferase which converts ornithine to pyrroline-5-carboxylate.

Hereditary cataract formation is also being studied in a strain of mice developed in our laboratory. These animals, known as Philly mice, develop osmotic cataracts by an as yet unknown mechanism.

Project Description:

Additional Personnel Engaged on Project:

Jin H. Kinoshita	Ph.D.	Scientific Director	NEI
Deborah A. Carper	B.A.	Biologist	LVR, NEI
Yoshio Akagi	M.D., Ph.D.	Visiting Scientist	LVR, NEI
Takashi Shiono	M.D.	Visiting Associate	LVR, NEI
Hirofumi Terubayashi	M.D.	Visiting Associate	LVR, NEI
Jane Millen	Ph..D.	Guest Worker	LVR, NEI

Objectives: To study the mechanism of cataract formation and diabetic pathology and to develop methods for its regulation.

Methods Employed: Sugar cataract formation can be induced in animals by either the use of a galactose-enriched diet or through the use of appropriate chemical agents such as streptozotocin. Lens metabolism can be studied in part through in vitro lens cultures in TC-199 bicarbonate buffer. Biochemical studies used for the purification of enzymes include column chromatography, polyacrylamide gel electrophoresis (PAGE), and high pressure liquid chromatography (HPLC). Immunohistochemical analysis includes the use of Ouchterloney plates, Laurell immunoelectrophoresis, and the coupled antibody DAB-PAP technique. Computational methods for enzyme analysis, inhibitor structure-activity studies, and basement membrane thickening studies require the use of the NIH PROPHET and DCRT computer systems.

Major Findings:

Aldose reductase has been purified from dog lens and its properties compared to enzyme from rat lens (RLAR) and human placenta (HPAR). The dog lens aldose reductase (DLAR) was purified by ammonium sulfate fractionation and subsequent affinity chromatography on Matrex Gel Orange A according to a procedure previously developed for the rat lens. This enzyme has an apparent lower affinity for the dye-liquid column than either RLAR or HPAR and like the rat enzyme appears to be closely associated with lens crystallin. On SDS-PAGE, the purified enzyme corresponds to the closely spaced doublet of ca 38K mw observed for rat lens aldose reductase. DLAR displayed substrate specificities which were similar to both RLAR and HPAR.

Antibodies were raised in goat against the purified DLAR proteins. These antibodies formed a single line of identity with aldose reductase from either rat lens, dog lens, human lens and monkey lens. With these antibodies against DLAR, aldose reductase was demonstrated to be present in the mural cells (pericytes) of trypsin digested dog retinal capillaries. The immunological presence of aldose reductase in retinal endothelial cells, however, could not be detected. In both diabetic and galactosemic dogs, a selective drop-out of retinal mural cells has been observed. In humans, this selective drop-out is considered a hallmark of early retinopathy. Previously the presence of aldose reductase has been observed by our laboratory only in human mural cells.

The presence of aldose reductase in diabetic and galactosemic rat lenses

has been investigated immunohistochemically using the peroxidase-anti peroxidase method with antibodies produced against purified RLAR. In the normal rat, aldose reductase appears to be localized in the epithelium and cortical fibers of the bow region with staining of the cortical fibers appearing to be greater in the young (50g) than older (200g) rat. In the diabetic rat, lens fibers in the anterior cortex stain more densely 2-3 weeks after injection of streptozotoein and by 5-6 weeks, dense, ring-shaped cortical staining can be observed especially near vacuoles. Similar but more intense distribution changes occur during the progression of galactose cataract. These findings reveal that, in both types of sugar cataracts, increased localization of immunoreactive aldose reductase appears in cortical fibers concomitant with their swelling, especially near the vacuoles that are formed.

The effect of aldose reductase on basement membrane thickening is also being investigated. We have observed that basement membrane thickening in diabetes can be produced in galactosemic rats and that this thickening in retinal capillary basement membrane can be prevented by two structurally unrelated aldose reductase inhibitors. These results suggest that aldose reductase may play a role in the biochemical mechanism in diabetes leading to the excessive formation of basement membranes. To study this possibility, we have looked for the presence of aldose reductase in the EHS tumor, a tissue that produces relatively large quantities of basement membrane. In this tumor we have found an enzyme with substrate characteristics similar to that of RLAR and HPAR. This enzyme can be inhibited by a variety of structurally diverse aldose reductase inhibitors. Moreover, the presence of the sugar alcohol dulcitol could be detected in tumor cells grown in galactose-fed mice.

The role of ornithine metabolism in gyrate atrophy was investigated in cultured rats lenses. Radiolabeled ornithine is accumulated into the lens via the energy dependent Ly^+ transport system and converted by ornithine amino transferase (OAT) to Δ_1-pyrroline-5-carboxylate (P-5-C). This conversion can be blocked through the use of the irreversible OAT inhibitor 4-aminohex-5-ynoic acid. The P-5-C intermediate in turn is converted by the enzyme P-5-C reductase to proline which is subsequently incorporated into lens proteins. Activation of the pentose pathway was observed with increased levels of P-5-C resulting from the OAT mediated metabolism of ornithine.

Significance to Biomedical Research and the Program of the Institute: Cataract is one of the major causes of blindness in the developing world. In the United States, vision loss due to cataract formation is one of the major health problems of the aging population. Moreover, in the diabetic population, vision loss due to cataract and retinopathy are significant. Through the study of aldose reductase, methods for the pharmocological control of these ocular diabetic complications may be developed.

Proposed Course: These studies will be continued. The biochemical properties of aldose reductase from human placenta, rat lens, dog lens and EHS tumor will be further compared so that the relationships can be more clearly understood. Detailed studies of the aldose reductase inhibitor site along with the mechanism of action these inhibitors will be conducted so that more potent and specific inhibitors may be developed.

NEI Research Program: Cataract--Diabetic and Metabolic Cataract

Publications:

Kador PF and Sharpless NE: Pharmacophore requirements of the aldose reductase inhibitor site. Mol Pharmacol 24:521, 1983.

Herrmann RK, Kador PF, and Kinoshita JH: Rat lens aldose reductase: Rapid purification and comparison with human placental aldose reductase. Exp Eye Res 37:467, 1983.

Akagi Y, Kador PF, Kuwabara T, and Kinoshita JH: Aldose reductase in human retinal mural cells. Invest Ophthalmol Vis Sci 24:1516, 1983.

Andrews JS, Leonard-Martin T, and Kador PF: Membrane lipid biosynthesis in the Philly mouse lens. Curr Eye Res 3:279, 1984.

Garadi R, Reddy VN, Kador PF, and Kinoshita JH: Membrane glycoproteins of Philly mouse lens. Invest Ophthalmol Vis Sci 24:1321, 1983.

Robison WG Jr, Kador PF, and Kinoshita JH: Retinal capillaries: Basement membrane thickening by galactosemia prevented with aldose reductase inhibitor. Science 221:1177, 1983.

Akagi Y, Yajima Y, Kador PF, Kuwabara T, and Kinoshita JH: Localization of aldose reductase in the human eye. Diabetes 33:562, 1984.

Kador PF and Kinoshita JH: Diabetic galactosemic cataracts. Ciba Foundation Symposium No. 106. Human Cataract Formation. 1984 (in press).

Kador PF, Robison WG Jr, and Kinoshita JH: Pharmacology of aldose reductase inhibitors. Annu Rev Pharmacol Toxicol (in press).

Shiono T, Kador P, and Kinoshita JH: Ornithine accumulation and metabolism in rat lens. Exp Eye Res (in press).

Kador PF and Sharpless NE: Pharmacophore requirements of the aldose reductase inhibitor site. 186th ACS National Meeting, Aug. 28, 1983. MEDI 10.

Kador PF, Millen J, Akagi Y, and Kinoshita JH: Dog lens aldose reductase: Purification and comparison with rat lens enzyme. Invest Ophthalmol Vis Sci 25 (Suppl):42, 1984.

Akagi Y, Kador PF, Shiono T, and Kinoshita JH: Aldose reductase distribution and activation in diabetic and galactosemic rat lens. Invest Ophthalmol Vis Sci 25(Suppl):136, 1984.

Millen J, Kador PF, Kinoshita JH, and Vogeli G: Aldose reductase and basement membrane production. Invest Ophthalmol Vis Sci 25(Suppl):154, 1984.

Shino T, Kador PF, and Kinoshita JH: Ornithine metabolism in the rat lens.

Invest Ophthalmol Vis Sci 25(Suppl):137, 1984.

Robison WG Jr, Kador PF, Akagi Y, and Kinoshita JH: Basement membrane thickening in ocular vessels of galactosemic rats prevented with aldose reductase inhibitors. Invest Ophthalmol Vis Sci 25 Suppl 66, 1984.

Carper D, Smith-Gill SJ, and Kioshita JH: Immunochemical localization of the 27,000 molecular weight lens polypeptide in mouse lenses using a monoclonal antibody. Invest Ophthalmol Vis Sci 25 Suppl 228, 1984.

Carper D, Smith-Gill SJ, and Kinoshita JH: Production and characterization of a monoclonal antibody to bovine β-crystallin. Curr Eye Res 3:501, 1984.

Carper D, Russell P, Shinohara T, and Kinoshita JH: Differential synthesis and mRNA translation of rat lens proteins during development. Exp Eye Res (accepted).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00136-12 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Metabolism of the Lens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Russell	Ph.D.	Research Chemist	LVR, NEI
Others:	S. Sato	M.D.	Guest Worker	LVR, NEI
	S.J. Schwab	M.S.	Chemist	LVR, NEI

COOPERATING UNITS *(if any)*
Department of Ophthalmology, Yale University (T. Reid).

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Lens and Cataract

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During lens development, there is a differential regulation of proteins and polypeptides. In rat, at least one beta crystallin polypeptide and one gamma crystallin are not present at birth but become prominent with age. In the human lens and in the monkey lens, the low molecular weight proteins also have alterations during aging. One of these low molecular weight proteins in the human lens increases with age but may be lacking in the soluble protein from cataractous zones. The monkey lens has been studied with particular reference to these proteins as well as some of the glycolytic enzymes. Glyceraldehyde-3-phosphate dehydrogenase, which decreases in cataract formation, has been isolated. This enzyme has many properties which are similar to the enzyme isolated from rabbit muscle.

Work has also been conducted on lens epithelial cells in tissue culture to investigate the influence of a retinoblastoma-derived growth factor on the growth of the cells.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: (1) to study development of the lens with particular reference to the formation of congenital cataracts; (2) to determine the sequence of events leading to cataract after an insult to the lens; and (3) to utilize a pure population of epithelial cells, the lens epithelium, to study biological processes of growth stimulation and suppression.

Methods Employed: Biochemical methods for analysis of proteins and cell growth were utilized. These included polyacrylamide gel electrophoresis, isoelectric focusing; mRNA extraction and cell free synthesis, and column chromatography. Immunological methods used included cell hybridization, enzyme linked immunoassay and western blotting.

Major Findings:

1. Developmental differences can be observed in rat lens during early postnatal development. These differences include the expression of a 27,000 dalton beta crystallin and the appearance of a gamma crystallin (γ_3). Both the beta polypeptide and the gamma crystallin are major components of the adult lens but are missing in the lens at birth. The beta component appears to be synthesized one or two days postnatally, whereas the gamma may not be expressed for about one week after birth. Thus, the early postnatal rat lens is in a transition from embryonic lens protein expression to an adult lens synthetic pattern. The developmental difference is only seen in some of the polypeptides and suggests that a differential regulation of gene expression is occurring at about the time of birth in the rat. This time would correspond to maturation of the retina also, but it is not known whether maturation of the retina is necessary for the regulation of these polypeptides in the lens.

2. The human low molecular weight proteins also undergo a change from the fetal to the adult lens. A 24,000 dalton component is more pronounced in the older lenses, whereas a 21,000 dalton protein is more prominent in the younger lenses. The significance of this change is not known, but it may suggest, by analogy to the rat, that a differential regulation of these low molecular weight components occurs in the young human lens.

3. Lens from young Rhesus monkeys display similar low molecular weight protein patterns to human. There is a clear difference in the monkey between the amount of 21,000 dalton protein in the nucleus and in the cortical area. This suggests the 21,000 dalton component is a predominant part of the embryonic lens and becomes less prominent with aging.

4. Rhesus monkey lenses have also been useful in studying one of the metabolic enzymes. Glyceraldehyde-3-phosphate dehydrogenase decreases in activity during cataract formation. This enzyme has been isolated from monkey lenses. The enzyme is a tetramer with a subunit molecular weight of 35,000. The enzyme has multiple isoelectric points in the basic region with the principal

band at pI=8.1. The enzyme is similar to the one isolated from rabbit muscle which is inactivated rapidly when cysteine in the active site is oxidized. This oxidation may be responsible for the inactivation of this enzyme in cataract formation.

5. Post-translational modifications of lens proteins in vitro and in lens culture are activated by calcium ions. These post-translational changes are similar to those observed with cataract formation. Use of an inhibitor of calcium-activated neutral proteinase, leupeptin, inhibited these modifications. This suggests these modifications are as a result of the action of a proteinase in the lens.

6. Cultures of mouse lens epithelial cells have been useful in studying the differences between cytolytic and cytostatic activities of macrophages and their regulation by prostaglandins. In addition, the lens cells have been used to study the growth stimulatory effect of retinoblastoma-derived growth factor. Growth factors secreted by the retina are known to influence lens differentiation, and, from the cultures of lens cells, a human growth stimulatory factor from retinoblastoma is starting to be characterized.

Significance to Biomedical Research and the Program of the Institute:
Studies of the development of the lens and the regulation of gene products is essential for investigating cataract formation, particularly congenital cataract formation. The study of the regulation of the low molecular weight protein in the primate lens may lead to an understanding of these important proteins in the human lens. In addition to the study of the development of the lens, once an insult that can cause cataract formation has occurred, the steps involved in this process and the post-translational changes in the lens proteins become important steps to examine and understand.

Proposed Course:

1. Investigation of human low molecular weight proteins will continue with emphasis on immunological methods.

2. Use of monkey lenses will also continue to determine the characteristics of these lenses and then to attempt the culture of the lens epithelial cells. A primate tissue culture cell line would be a particularly useful tool in studying the lens.

3. Studies will continue on the development of the lens and the relationship of the retina and retinal factors to the regulation of the proteins in the lens.

NEI Research Program: Cataract--The Normal Lens

Publications:

Tarsio JF, Rubin NA, Russell P, Gregerson DS, and Reid TW: Growth stimulatory effects of retinoblastoma-derived growth factors and other mitogens on Nakano mouse lens epithelial cells. Exp Cell Res 146:71, 1983.

Mochizuki M, Zigler JS Jr, Russell P, and Gery, I: Serum proteins neutralize the toxic effect of lysophosphatidyl choline. Current Eye Res 2:621, 1983.

Mochizuki M, Zigler JS Jr, Russell P, and Gery, I: Cytostatic and cytotoxic activities of macrophages: Regulation by prostaglandins. Cell Immunol 83:34, 1984.

Russell P: In vitro alterations similar to post translational modifications of lens proteins. Invest Ophthalmol Vis Sci 25:209, 1984.

Carper D, Russell P, Shinohara T, and Kinoshita JH: Differential synthesis of rat lens proteins during development. Exp Eye Res (in press).

Russell P, Schwab SJ, and Garland D: The characteristics of monkey lens glyceraldehyde-3-phosphate dehydrogenase. Lens Res (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00105-05 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Structure and Composition of Lens Crystallins with Respect to Cataractogenesis

PRINCIPAL INVESTIGATOR *(List other profassional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Samuel Zigler, Jr.	Ph.D.	Research Biologist	LVR, NEI
Others:	Venkat N. Reddy	Ph.D.	Visiting Scientist	LVR, NEI
	Jose L. Lepe-Zuniga	M.D.	Visiting Fellow	LVR, NEI

COOPERATING UNITS *(if any)*
Jules Stein Eye Institute, UCLA Medical School (J. Horwitz); Division of Biology, Kansas State University (L. Takemoto).

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Lens and Cataract

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structures of lens crystallins and mechanisms responsible for post-synthetic modifications observed in these long-lived proteins during aging and cataractogenesis have been investigated. Three distinct classes of human γ-crystallin have been identified and isolated. All three species have been shown to undergo a similar pattern of age-related modification in net charge. Evidence supporting the hypothesis that lipid peroxidation is responsible for the initiation of cataract in diseases involving retinal degeneration has been generated from studies on the Royal College of Surgeons rat model. Investigation of the effects of activated states of oxygen on the lens suggests that under conditions of generation outside the lens, the hydroxyl radical, an extremely strong oxidant, is less damaging to the organ cultured lens than is hydrogen peroxide, a much weaker but longer-lived species.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: (1) Elucidation of biochemical mechanisms responsible for post-synthetic modifications to lens crystallins; (2) Isolation and characterization of the human γ-crystallins; and (3) Investigation of oxidative mechanisms which may be involved in senile cataractogenesis.

Methods Employed: Conventional protein chemical techniques of chromatography, electrophoresis, and isoelectrofocusing are employed. Additionally, lens proteins are studied immunochemically using specific antisera and by high pressure liquid chromatography (HPLC) and fluorescence techniques. Lens organ culture experiments utilized rat lenses, with active transport and membrane permeability parameters being used to measure the effect of various stresses on the cultured lenses.

Major Findings:

1. Studies have continued on the hypothesis that lipid peroxidation products initiate the posterior subcapsular cataracts associated with retinal degenerative diseases. Using the Royal College of Surgeons (RCS) rat, the presence of lipid peroxidation in the degenerating retinal tissue has been established. Lens damage in the model system appears to occur only during the time period in which peroxidation products are present in the eye. Both the lenses from dystrophic rats and normal rat lenses exposed to peroxidation products in vitro show increased permeability to cations.

2. Since compositional changes in human low molecular weight crystallins have been associated with senile cataract, we have initiated studies to isolate and characterize these protein species. Three distinct γ-crystallin fractions have been identified and separated chromatographically from young lenses. All three undergo similar post-translational modifications in charge. The relative amounts of the three species vary with age.

3. The relative effects of the activated species of oxygen, the hydroxyl radical (OH·) and hydrogen peroxide (H_2O_2), on lenses in organ culture have been investigated using a xanthine oxidase system to generate H_2O_2 and superoxide. Lenses, normally damaged by exposure to this system, were protected from such damage by the addition of iron, which rapidly led to generation of the extremely strong oxidant OH· via the reduction of H_2O_2. This suggests that while intracellularly OH· is an extremely toxic agent, it may be less damaging than H_2O_2 when generated outside the lens.

4. Cell cultures have been shown to grow irregularly in culture media containing the buffer N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid (HEPES). Our recent studies have demonstrated that the growth of cell cultures, including lens epithelial cells, in such media is affected by the extent to which the medium has been exposed to light. A cytotoxic product, as yet unidentified, is apparently produced from HEPES via a photooxidation process in which riboflavin acts as a sensitizer.

Significance to Biomedical Research and the Program of the Institute: The crystallins are the primary constituents of the lens and are ultimately responsible for its transparency. Our studies are directed towards a fuller understanding of the structure and function of these proteins in the normal lens and how these parameters are altered during aging and cataractogenesis. It is hoped that these studies may contribute to the development of means of retarding or preventing the development of cataracts.

Proposed Course: The following studies are underway or are planned for the upcoming year.

1. Continuation of work on the human γ-crystallins with emphasis on the structural modifications and compositional changes which occur during aging and particularly during development of cataract.
2. Further studies directed toward elucidation of mechanisms responsible for oxidation of lens proteins in vivo.
3. HPLC analysis of microdissected portions of cataractous lenses obtained at surgery.

NEI Research Program: Cataract--Senile Cataract

Publications:

Zigler JS Jr, Bodaness RS, Gery I, and Kinoshita JH: Effects of lipid peroxidation products on the rat lens in organ culture: A possible mechanism of cataract initiation in retinal degenerative disease. Arch Biochem Biophys 225:149, 1983.

Zigler JS Jr, and Goosey JD: Singlet oxygen as a possible factor in human senile nuclear cataract development. Curr Eye Res 3:59, 1984.

Mochizuki M, Zigler JS Jr, Russell P, and Gery I: Serum proteins neutralize the toxic effect of lysophosphatidyl choline. Curr Eye Res 2:621, 1983.

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Bodaness RS, LeClair M, and Zigler JS Jr: An analysis of the H_2O_2-mediated crosslinking of lens crystallins catalyzed by the heme-undecapeptide from cytochome c. Arch Biochem Biophys 231:461, 1984.

Takemoto LJ, Hansen JS, Zigler JS Jr, and Horwitz J: Characterization of polypeptides from human nuclear cataracts by Western blot analysis. Exp Eye Res (in press).

Zigler JS Jr, and Reddy VN: Effects on the cultured lens and on lens crystallins of oxidants generated by a xanthine oxidase system. Invest Ophthamol Vis Sci 25

(Suppl):139, 1984.

Datiles MB, Stone H, Hu T-S., Amsbaugh DF, Zigler JS Jr, and Kinoshita JH: Congenital cataracts in guinea pigs. Invest Ophthalmol Vis Sci 25(Suppl):140, 1984.

Takemoto LF, Hansen JS, Zigler JS Jr, and Horwitz J: Monospecific antisera to human lens crystallins: Preparation and use in Western blot analysis of polypeptides from the human cataractous lens. Invest Ophthalmol Vis Sci 25(Suppl):80, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00148-11 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclic Nucleotides and Vision

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gerald J. Chader	Ph.D.	Chief	LVR, NEI
Others:	C. Lal Kapoor	Ph.D.	Guest Worker	LVR, NEI
	Susan Gentleman	Ph.D.	Expert	LVR, NEI
	R. Theodore Fletcher	M.S.	Chemist	LVR, NEI
	Robert L. Somers	B.S.	Chemist	LVR, NEI

COOPERATING UNITS *(if any)*
Section on Ophthalmology, School of Veterinary Medicine, University of Pennsylvania (G. Aguirre); Department of Anatomy, Erasmus University, Rotterdam, The Netherlands (S. Sanyal)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	1.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The roles of cyclic nucleotides and protein kinases in normal vision and in retinal degeneration have been further examined: 1. A new calcium phospholipid-dependent protein kinase (C-kinase) has been identified in retinal photoreceptors. 2. Protein tyrosine kinase has also been partially characterized in human retinoblastoma cells. 3. From genetic crosses of rd and rds mouse mutants we have found that the genes affect retinal cyclic nucleotide metabolism in different manners but each ultimately leads to photoreceptor cell death. 4. In a case of human choroideremia, aberrently high levels of cyclic AMP were found in the pigment epithelium-choroid unit and thus may be involved in the etiology of the disease.

PHS 6040 (Rev 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

Merlyn Rodrigues	M.D., Ph.D.	Head, Section on Clinical Eye Pathology	LOP, NEI

Objectives: (1) To study the role of cyclic nucleotides and their enzymes of metabolism in normal retinas and those with retinal degeneration. (2) To study the general role of protein phosphorylation in functioning of the retinal photoreceptor-pigment epithelial complex and also in the inner layers of the neural retina.

Methods Employed: Standard biochemical and neurochemical techniques are utilized.

Major Findings:

1. A calcium phospholipid-dependent protein kinase (C-kinase) activity was detected in the soluble fraction of bovine rod outer segments (ROS). The enzyme required calcium, phosphatidylserine and diacylglycerol for maximal activity. In the presence of calcium and PS, C-kinase endogenously phosphorylated proteins with molecular weights of 95,000, 31,000, 21,000, 19,000, 18,000, 16,000, 14,000 and 11,000. Addition of diolein in the reaction mixture further enhanced the endogenous phosphorylation of proteins. Retinal was found to inhibit the phosphorylation of endogenous proteins by C-kinase in a concentration-dependent manner. Half-maximal inhibition of enzyme activity was obtained at a retinal concentration of about 10 μM. These results suggest that calcium, phospholipids, and the C-kinase enzyme play an important role in the functional regulation of rod photoreceptor cells and with retinal, may be directly involved in the visual process.

2. Protein tyrosine phosphorylation (PTK) is involved in the processes of transformation in many cells. We have found PTK activity to be high in Y-79 human retinoblastoma cells and in normal postmortem human retina. In Y-79 cells, PTK activity increases during the first 8 hrs after the initiation of growth by "splitting" the cells. PTK activity is increased in relation to both endogenous and exogenous substrates. Addition of retinoic acid to the culture medium dramatically inhibited the rise in PTK activity. Addition of Retinoblastoma-Derived Growth Factor (RDGF) to the medium increased endogenous phosphorylation of protein but not of exogenous protein substrates. Thus, Y-79 cells exhibit a very active PTK activity, retinoic acid blocks activation of PTK, and RDGF enhances endogenous phosphorylation apparently by altering natural protein substrates.

3. In retinas of the rds and rd mouse mutants which demonstrate photoreceptor dysplasia, cyclic GMP phosphodiesterase activity is low, even in the early postnatal period. Cyclic GMP is abnormally high in the rd retina but

abnormally low in the rds retina. Other enzymes of the cyclic GMP metabolic system are also affected. Genetic crosses of rd and rds animals indicate that the two gene defects are separate but that both ultimately result in photoreceptor cell death.

4. In an early case of human hereditary choroideremia, retinal cyclic nucleotide levels were found to be minimally altered. However, abnormally high cyclic AMP levels were observed in samples of the pigment epithelium-choroid of this patient. No marked changes were observed in cyclic GMP content. Since choroideremia is primarily a disease of the choroid-pigment epithelial unit, the abnormal cyclic GMP level may indicate a primary defect in cyclic AMP metabolism in this disease.

Significance to Biomedical Research and the Program of the Institute: Only by thoroughly defining both normal and abnormal functioning of a tissue can one understand a disease process. We hope ultimately to be able to slow or even stop the degenerative process in the retinas of animal models of RP and finally in human retinitis pigmentosa itself.

Proposed Course: Experimentation will continue on control mechanisms in the normal retina and in genetic diseases of the retina.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Winkler B, Fletcher R, and Chader G: Effects of diamide on cyclic nucleotide levels in rat retina. Invest Ophthalmol Vis Sci 25:461, 1984.

Sanyal S, Fletcher R, Liu YP, Aguirre G, and Chader G: Cyclic nucleotide metabolism in photoreceptor dysplasia: developmental patterns in the rds mouse. Exp Eye Res 38:247, 1984.

Gentleman S, Martensen TM, DiGiovanna JJ, and Chader GJ: Protein tyrosine kinase and protein phosphotyrosine phosphatase in normal and psoriatic skin. Biochim Biophys Acta 798:53, 1984.

Gentleman S, Russell P, and Chader G: Protein phosphorylation studies in Y-79 retinoblastoma cells. Invest Ophthalmol Vis Sci 25(Suppl):261, 1984.

Kapoor CL and Chader GJ: Endogenous phosphorylation of retinal photoreceptor outer segment proteins by calcium phospholipid-dependent protein kinase. Biochem Biophys Res Commun (in press).

Rodrigues MM, Ballintine EJ, Wiggert BN, Lee L, Fletcher RT, and Chader GJ: Choroideremia: A clinical, electron microscopic and biochemical report. Ophthalmology (in press).

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Chader GJ: Biochemical studies of retinal degeneration in animal models and in the human. Klin Monatsbl Augenheilkd (in press).

Chader GJ, Aguirre GA, Sanyal S, Acland G, and Stramm L: Mechanisms of hereditary visual cell disease. In Hereditary and Visual Development, Hilfer R and Sheffield J, editors. Berlin, Springer-Verlag (in press).

Chader GJ, Aguirre GA, and Sanyal S: Studies on animal models of retinal degeneration. In Retinal Diseases: Biomedical Foundation and Clinical Management, Tso, MO, editor. Philadelphia, PA, J.B. Lippincott (in press).

Gierschik P, Simons C, Woodard C, Somers RL, and Spiegel A: Antibodies against a retinal guanine nucleotide binding protein cross-react with a single plasma membrane in non-retinal tissues. Biochem Biophys Res Commun (in press).

Shichi H, Yamamoto K, and Somers RL: GTP binding protein: Properties and lack of activation by phosphorylated rhodopsin. Vision Res (in press).

Somers RL and Klein DC: Rhodopsin kinase activity in the mammalian pineal gland and other tissues. Science (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00124-04 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of the Retina and Pigment Epithelium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gerald J. Chader	Ph.D.	Chief	LVR, NEI
Others:	Shay-Whey M. Koh	Ph.D.	Staff Fellow	LVR, NEI
	Athanassios P. Kyritsis	M.D.	Visiting Fellow	LVR, NEI
	Paul Madtes	Ph.D.	Staff Fellow	LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Characteristics of metabolism and function of the neural retina (NR) and pigment epithelium (PE) are studied in culture cells and in freshly dissected tissue. 1. Several new neurotransmitter/neuromodulator receptors have been identified and partially characterized in retina, pigment epithelial cells, and retinoblastoma cells in culture. These include receptors for vasoactive intestinal peptide, glucagon prostaglandin E_1, histamine, and isoproterenol. These all operate through activation of adenylate cyclase activity. 2. Human retinoblastoma cells can be induced to differentiate in culture. Agents such as cyclic AMP, butyrate, and retinoic acid can be used to induce differentiation, stop growth, and cause cell death. 3. GABA, besides being a classical neurotransmitter, appears to exert a marked trophic influence on early retinal development.

Project Description:

Additional Personnel Engaged on Project:

C. Lal Kapoor	Ph.D.	Guest Worker	LVR, NEI

Objectives: To better understand the general metabolism of the retina-pigment epithelial unit and to apply this information to diseases which affect these tissues.

Methods Employed: PE and retinal cell cultures were maintained from tissues of the chick embryo. When appropriate, fresh tissues were dissected from the eye aided by a stereomicroscope. Retinoblastoma cells were maintained in culture under standard conditions. Biochemical analyses were performed using standard assay procedures as adapted in our laboratory for the particular ocular tissue.

Major Findings:

1. The pigment epithelium (PE) is not normally thought of as a hormonal or neurotransmitter target tissue. We have found, however, that PE cells in culture respond to vasoactive intestinal peptide (VIP), glucagon, TSH, PGE, histamine, and β-adrenergic agonists with a substantial increase in the intracellular concentrations of cyclic AMP. Retinal glial (Muller) cells have also been successfully grown in culture and were found to respond to VIP. This is the first demonstration of a direct effect of a neuropeptide on Muller cells.

2. Most cancer treatment modes deal with methods of causing the death of the cancer cell. Another, better but more difficult method would be to cause the differentiation of the tumor cell to a more normal state. We are following both of these courses in our studies on human Y-79 retinoblastoma cells.

The effects of butyrate, retinol, and retinoic acid were tested on growth and differentiation of human Y-79 retinoblastoma cells in monolayer cultures. Treatment with 4 mM butyrate resulted in marked growth inhibition of cells, due mostly to increased death rate. The effect was greater in serum-supported cultures in which the proliferation rate is higher than in the serum-free, defined medium in which the cells are differentiated and the growth rate is slow, suggesting a cell-cycle specific action of this substance. Moreover, butyrate induced morphological changes in the viable cells, consisting of an elongated appearance of the cells and retraction of long processes formed in the serum-free supported cultures. Retinol at 20 μM also affected the cell viability both in serum-containing and in serum-free culture medium. Retinoic acid at 50 μM induced reversible growth inhibition of cells growing in serum-containing medium, and cell death in the defined-medium supported cultures. Combination of 0.5 mM butyrate with 50 μM retinoic acid resulted in an enhanced inhibition of growth in an apparently synergistic fashion. It would be interesting to determine if similar effects exist in vivo. This is particularly appealing since the concentrations of the two effectors used in our study are far below the levels at which, individually, they are toxic. In any event,

our results demonstrate that physiological compounds such as butyric acid and retinoids have antitumor activity in human retinoblastoma cells in culture. If similar effects are found in vivo, these agents could be useful chemotherapeutic drugs for retinoblastomas, particularly when used in combination, since the side effects of these naturally occurring substances most probably would be much less severe than those caused by the currently used antineoplastic agents.

3. The binding of GABA and its analog, nipecotic acid, continues to be investigated in developing and mature retina. Chloride ion has been found to interact with the GABA receptor and to mask high affinity binding sites. Much work now indicates that GABA exerts a trophic influence on retinal development.

Significance to Biomedical Research and the Program of the Institute: The pigment epithelium is an important cell layer which acts as a partner with the retina in the visual process. Understanding the basic factors which promote differentiation and the basic factors which control PE and retinal cell metabolism should aid us in better understanding dysfunction of the PE-retina unit.

Proposed Course: The metabolic capabilities of the retinal cells will be further investigated. Correlations with specific disease of the PE-retina unit will be made.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Koh SW and Chader GJ: Agonist effects on the intracellular cyclic AMP concentration of retinal pigment epithelial cells in culture. J Neurochem 42:287, 1984.

Koh SW and Chader G: Retinal pigment epithelium in culture demonstrates a distinct β-adrenergic receptor. Exp Eye Res 38:7, 1984.

Kyritsis A, Koh SW, and Chader GJ: Modulators of cyclic AMP in monolayer cultures of Y-79 retinoblastoma cells: Partial characterization of the response with VIP and glucagon. Curr Eye Res 3:339, 1984.

Kyritsis A, Tsokos M, Triche T, and Chader G: Retinoblastoma: Origin from a primitive neuroectodermal cell? Nature 307:471, 1984.

Kyritsis A, Tsokos M, and Chader G: Attachment culture of human retinoblastoma cells: Long-term culture conditions and effects of dibutyryl cyclic AMP. Exp Eye Res 38:411, 1984.

Barbehenn EK, Masterson E, Koh SW, Passonneau JV, and Chader GJ: An examination of the efficiency of glucose and glutamine as energy sources for cultured chick pigment epithelial cells. J Cell Physiol 118:262, 1984.

Koh SW and Chader GJ: Chick retinal pigment epithelium: A new culture system for studying H-2 histamine receptors. FEBS Lett 164:277, 1983.

Koh SW, Kyritsis A, and Chader GJ: Interaction of neuropeptides and cultured glial (Muller) cells of the chick retina: Elevation of intracellular cyclic AMP by vasoactive intestinal peptide and glucagon. J Neurochem 43:199, 1984.

Kyritsis AP, Tsokos M, Triche T, Kapoor CL and Chader GJ: Differentiation of human retinoblastoma (Y-79) cells in monolayer culture. Invest Ophthalmol Vis Sci 25(Suppl):108, 1984.

Madtes P and Redburn DA: Intraocular injections of nipecotic acid produce a preferential block of neuronal 3H-GABA accumulation in adult rabbit retina. Invest Ophthalmol Vis Sci 24:84, 1983.

Madtes P and Redburn DA: Synaptic interactions of the GABA system during postnatal development in rabbit retina. Brain Res Bull 10:741, 1983.

Madtes P and Redburn DA: GABA as a trophic factor during development. Life Sci 33:979, 1983.

Redburn DA, Massey SC, and Madtes P: The GABA uptake system in rabbit retina. In Glutamine, Glutamate, and GABA in the Central Nervous System, Hertz L, Kvanne E, McGeer EG, Schousboe A, editors. New York, Alan Liss, Inc, 1983, pp. 273-286.

Madtes P: Chloride ions preferentially mask high-affinity GABA binding sites. J Neurochem (in press).

Koh SW and Chader GJ: Elevation of intracellular cyclic AMP and stimulation of adenylate cyclase activity by VIP and glucagon in the retinal pigment epithelium. J Neurochem (in press).

Kyritsis A, Joseph G, and Chader, G: Effects of butyrate, retinol and retinoic acid on Y-79 retinoblastoma cells growing in monolayer cultures. J Natl Cancer Inst (in press).

Kapoor CL, Kyritsis AP, and Chader GJ: Alteration in gene expression at the onset of human Y-79 retinoblastoma cell differentiation. Neurochem Int (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00146-03 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Proliferation in Diabetic Retinopathy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Leonard M. Hjelmeland Ph.D. Expert LVR, NEI

COOPERATING UNITS *(if any)*
Reproductive Research Branch, NICHD (A. Chrambach); Surgical Neurology Branch, NINCDS (J. Bressler); Laboratory of Developmental Biology and Anomolies, NIDR (G. Grotendorst)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.0	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research is being conducted on the biological mechanisms which control chemotaxis and proliferation of several cell types in diabetic retinopathy which include fibroblasts, glia pericytes, and vascular endothelial cells. Using wound repair as a general model, we have demonstrated that glial cells exhibit chemotaxis to platlet-derived growth factor (PDGF) in a fashion similar to that described in previous reports on smooth muscle and fibroblasts. In addition, we are characterizing and purifying a potent chemoattractant present in retina which attracts glial cells and possibly endothelial cells.

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To elucidate the sequence of cellular and biochemical events which lead to the development of neovascularization and fibroplasia in proliferative diabetic retinopathy. To isolate any soluble macromolecules which may mediate these events and develop methods to alter the course of the disease.

Methods Employed: Primary cell cultures of glial cells, pericytes, and vascular endothelial cells are established from cow and rat retina and brain tissues. The cells themselves are tested for chemotaxis to a variety of macromolecules thought to be involved in wound repair such as platelet-derived growth factor; in addition, chemotaxis factors produced by the bovine retina are examined for their activity on each of the individual cell types. These factors are currently being purified by combinations of affinity chromatography on heparin sepharose and size exclusion chromatography on high performance liquid chromatography.

Major Findings:

1. Astrocytes from the postnatal rat brain have been shown to migrate toward gradients of platlet-derived growth factor and fibronectin. This finding establishes both the role of PDGF and wound repair in gliosis, and at the same time demonstrates the fact that non-mesodermal cells can participate in the wound repair response, at least in the central nervous system.

2. Conditioned media from transformed human glial cells contain chemoattractants for endothelial cells. In order to understand angiogenesis in the central nervous system, it is important to compare, for example, cortex with retina. Since attractants for endothelial cells have been isolated from bovine retina, we are comparing such material with attractants produced by human gliomas.

3. We have demonstrated the presence of a macromolecular chemoattractant in bovine retina and have purified this factor. This material is a slightly acidic (PI 6-7) species with a molecular weight of approximately 18,000 daltons as determined by SDS polyacrylamide gel electrophoresis. Crude soluble protein from bovine retina contains enough chemoattractant to display a half-maximal activity at 5 micrograms/ml. When purified to homogeneity, this protein exhibits a specific activity of at least 1,000,000 units/mg, indicating an overall purification of approximately 10-20,000 fold. This purification is accomplished by heparin agarose affinity chromatography in combination with high performance gel exclusion chromatography. Beginning with 5 grams of soluble protein from 500 bovine retinas, we are able to prepare approximately 500 micrograms of the purified factor on a routine basis. In experiments performed to evaluate the biological activity of this protein, we have recently shown this protein to be a growth factor which stimulates the division of vascular smooth muscle, astroglia, and endothelial cells at concentrations below 1 nanogram/ml. Based on these biological activities and comparisons of

several physical properties such as the molecular weight, isoelectric point, high affinity binding to heparin, and amino acid composition, we have tentatively identified this protein as the acidic fibroblast growth factor, thought to be the principle mitogen for connective tissue found in the central nervous system.

Significance to Biomedical Research and the Program of the Institute: Establishing a sequence of cellular and biochemical events which lead to proliferative retinopathy could lead to the rational design of procedures or drugs which could modify the course of this disease.

Proposed Course: Glial cells, pericytes, and capillary endothelial cells will be obtained in primary culture from bovine retina. Conditioned media from these cells will be examined for factors that are chemotactic for any of the other cell types. Such factors will be purified to the extent possible and characterized. Using retina culture, a physiological role of such factors will be explored by subjecting the retina to a variety of insults, and examining the response of the tissue in terms of production of specific chemoattractants. The acidic fibroblast growth factor which has just been purified will be prepared in quantities sufficient for sequence analysis and cloning studies. It is hoped that the cloning studies will eventually produce larger quantities of the human protein.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications:

Hjelmeland LM and Chrambach A: Solubilization of functional membrane bound receptors. In Receptor Biochemistry in Methodology, Venter CJ and Harrison L, editors. New York, Alan R. Liss, 1984, pp. 35-46.

Mackenzie PI, Hjelmeland LM, and Owens IS: Purification of mouse liver UDP glucoronosyltransferase. Arch Biochem Biophys 231:487, 1984.

Hjelmeland LM and Chrambach A: Solubilization of functional membrane proteins. In Methods in Enzymology, Jacoby W, editor. New York, Academic Press, 1984, pp. 305-318.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00195-01 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Molecular Genetics of δ-Crystallin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John M. Nickerson	Ph.D.	Senior Staff Fellow	LVR, NEI
Others:	Joram Piatigorsky	Ph.D.	Chief, Laboratory of Molecular and Developmental Biology	LMDB, NEI
	Teresa Borras	Ph.D.	Staff Fellow	LMDB, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular genetics of the lens are being investigated through the study of δ-crystallin. This protein and its gene and mRNA are being investigated at the levels of gene organization, expression, and nucleotide sequence. There are two genes for δ-crystallin in the chicken genome. The genes are located together in one 30 Kb (kilobase) locus and are 4.2 Kb apart. They are oriented in the same direction for transcription. cDNAs corresponding to only the first gene have been found, suggesting that mRNA from gene 1 is much more abundant than from gene 2. It remains to be shown that gene 2 produces a functional mRNA. A full-length cDNA clone has been obtained and sequenced. The mRNA for δ-crystallin is 1572 bases in length (excluding the Poly(A) tail) and encodes a 447 amino acid polypeptide. The promoters from genes 1 and 2 have been sequenced and compared. There are several structural differences that might account for the differences in transcriptional activity of genes 1 and 2.

PHS 6040 (Rev. 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project:

James W. Hawkins	Ph.D.	Fogarty Fellow	LMDB, NEI
Eric Wawrousek	Ph.D.	Staff Fellow	LMDB, NEI
Aida Wakil	B.S.	Biologist	LMDB, NEI

Objectives: The major objective of this project is to understand the structure, organization, function, and developmental expression of δ-crystallin and its genes. Particular attention is given to understanding gene expression under conditions likely to give rise to cataracts.

Methods Employed: Standard methods of recombinant DNA technology are employed.

Major Findings:

1. The entire mRNA and amino acid sequences have been obtained for δ-crystallin.

2. These sequences are not homologous to any other known sequences.

3. Only one mRNA type has been found. Other cDNA clones have been obtained, but all appear identical to the one known mRNA type.

4. There is a possibility that the one mRNA could generate the two polypeptides that compose δ-crystallin protein via the use of each of the first two methionine codons as an initiator of protein synthesis.

5. There are two non-allelic genes in one locus separated by 4.2 Kb.

6. Gene 1, the 5' gene, produces the one mRNA which gives rise to all the cDNAs found so far. The nucleotide sequence of a gene 2 mRNA is distinctly different from the gene 1 product.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the gene structure and expression of a lens protein present in such large amounts in a stage and tissue specific manner is fundamental to understanding normal lens development and might give rise to strategies to influence or control synthesis and to ameliorate cataractogenesis.

Proposed Course:

1. Complete the nucleotide sequence of the entire 30 Kb gene locus.

2. Analyze the translation products of mRNA produced *in vitro* from the full length cDNA subcloned into the SP-6 vector-promotor system. This experiment will test for the possible use of each of the first two met codons as an initiator of protein synthesis.

NEI Research Program: Cataract--The Normal Lens

Publications:

Piatigorsky J, Treton JA, King CR, Nickerson JM, Carper D, Shinohara T, Inana G, Hejtmancik JF, and Norman B: A molecular genetic approach to vision research: crystallin gene expression in the lens. Ophthal Ped Gen 3:61, 1983.

Nickerson JM and Piatigorsky J: The complete sequence for a chicken δ-crystallin cDNA. Proc Natl Acad Sci USA 81:2611, 1984.

Nickerson JM, Hawkins JW, Borras T, and Piatigorsky J: cDNAs and genes of chicken δ-crystallin. J Cell Biol 97:135A, 1983.

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Borras T, Nickerson JM, Hawkins JW, Das G, and Piatigorsky J: δ-Crystallin gene expression in the embroynic chicken lens. Invest Ophthalmol Vis Sci 25(Suppl):152, 1984.

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Das GC, Nickerson JM, Hawkins JW, and Piatigorsky J: Expression of δ-crystallin genes in vitro. Fourth Annual Congress for Recombinant DNA Research. DNA (in press).

Hawkins JW, Nickerson JM, Sullivan MA, and Piatigorsky: The chicken δ-crystallin gene family. Two genes of similar structure in close chromosomal approximation. J Biol Chem 259:9821, 1984.

Piatigorsky J, Nickerson, JM, King CR, Inana G, Hejtmancik JF, Hawkins JW, Borras T, Shinohara T, Wistow G, and Norman B: Crystallin genes: Templates for lens transparency. In Human Cataract Formation, Nugent J, editor. Elsevier, Ciba Foundation Symposium No. 106, Pitman Publishers (in press).

Piatigorsky J, Chepelinsky AB, Hejtmancik JF, Borras T, Das GC, Hawkins JW, Zelenka PS, King CR, Beebe DC, and Nickerson JM: Expression of crystallin gene families in the differentiating eye lens. In Molecular Biology of Development, Davidson E and Firtel RA, editors. New York, Cetus-UCLA Symposium, Alan R. Liss (in press).

Borras T, Nickerson JM, Hawkins JW, and Piatigorsky J: Structural evidence for differential promotor activty of the linked δ-crystallin genes in the chicken. EMBO Journal (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00197-01 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line betwaen the borders.)*
Linkage Markers for Human Eye Diseases of Genetic Origin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: John M. Nickerson Ph.D. Senior Staff Fellow LVR, NEI

COOPERATING UNITS *(if any)*
Division of Human Genetics, University of Maryland, School of Medicine
(J. Boughman)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recombinant DNA technology offers new ways to study genetic eye disease. One important use is in the mapping of the diseases on the human genome. We are in the preliminary stages of finding a linkage marker for ARRP (Autosomal recessive retinitis pigmentosa). Approximately 500 different family pedigrees from the National Registry of the National Retinitis Pigmentosa Foundation have been examined to identify the best families suitable for a linkage study. The most important criteria are: pedigrees that are clearly autosomal recessive and with a large number of affected siblings or with a large number of siblings (affected plus unaffected). It appears that a sufficient number of suitable families exist that linkage could be detected at a recombinantion fraction of 0.2 or less. A number of DNA probes have been collected that detect highly polymorphic variations in human DNA.

Project Description:

Additional Personnel Engaged on Project:

Diane Borst	Ph.D.	Guest Worker	LVR, NEI
Muriel Kaiser-Kupfer	M.D.	Head, Section on Ophthalmic Genetics and Pediatric Ophthalmology	CB, NEI

Protocol Number: 78-EI-01

Objectives: The objectives of this project are to obtain a linkage marker for ARRP and to map the disease locus/loci for ARRP on the human genome.

Methods Employed: Standard methods of DNA blot hybridization are used to analyze human genotypes for each marker polymorphism. Human DNA is obtained from white blood cells. Polymorphisms in red blood cell antigens and serum proteins are obtained by standard techniques. Linkage is assessed via sequential analysis of likelihood ratios by use of the computer program, LIPED.

Major Findings: Sufficient pedigrees have been identified to carry out a linkage study for ARRP. This is shown in tabular form below:

# of Affected Siblings per Family	# of Informative Families needed For a Complete Study	# of Families in National Registry	% of Total Needed
4	5 to 6	4	57-80%
	or		
3	35	8	23%
	or		
2	200	16	8%
			Sum= 98%-111%

Significance of Biomedical Research and the Program of the Institute: The identification of a linkage marker for autosomal recessive retinitis pigmentosa will provide the location of the gene for ARRP. A tightly linked marker could be used for antenatal diagnosis. Such a marker defines a region of DNA (1-10 million basepairs) which contains the gene(s) for ARRP, and with further studies including the cloning of this region, counterscreening techniques, and/or expression of the genes in the locus, the specific gene and the lesion(s) in it that cause ARRP might be identified.

Proposed Course:

1. Contact families suitable for this study.
2. Obtain blood samples for analysis from all family members, perform clinical evaluation of patients to verify diagnosis.
3. Isolate DNA from white blood cells.

4. Perform Southern blot analysis.
5. Perform red blood cell polymorphism analysis.
6. Perform serum protein polymorphism analysis.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 EY 00196-01 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetics of IRBP

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: John M. Nickerson Ph.D. Senior Staff Fellow LVR, NEI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structure, organization, expression and evolution of IRBP (Interphotoreceptor retinol binding protein) gene(s) and mRNA(s) are being investigated. cDNA clones for IRBP are being constructed and identified. Two different cDNA libraries have been obtained. One library is an expression library in the λgt11 vector system and is being screened with antibodies against IRBP. Several prospective positive clones have been obtained from the primary screening of this library, these clones are being re-examined to eliminate false-positives. A second library has been obtained that can be screened with oligonucleotide probes, the sequence of which is based on the known amino acid sequence of the N-terminus of IRBP.

Project Description:

Additional Personnel Engaged on Project:

David Barrett	M.D.	Staff Fellow	LVR, NEI
Barbara Wiggert	Ph.D.	Research Chemist	LVR, NEI
Gerald Chader	Ph.D.	Chief	LVR, NEI

Objectives: The isolated IRBP cDNA clones will be used as molecular probes to study gene regulation during the development of the retina and eyes. The cloned cDNA(s) will allow the isolation of gene clones. The exon-intron structure of the gene, its DNA sequence, and the deduced amino acid sequence will contribute to the understanding of the regulation, expression, and function of this protein.

Methods Employed: Conventional techniques for cloning and analysis of nucleic acids are used.

Major Findings:

1. cDNA libraries in λgt10 and tg11 vector systems have been obtained.
2. Immunoblot technique has been established and the assay has been validated.
3. Several positive clones have been obtained from primary screening of the expression library.
4. The N-terminal sequence of bovine IRBP is not homologous to other amino acid sequences in available computer databases, and thus may be suitable for predicting an oligonucleotide sequence for use as a nucleic acid probe for screening the two available cDNA libraries.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the mRNA, amino acid, and gene and sequences of IRBP is fundamental to understanding normal retina development and function.

Proposed Course for FY 1985:

1. Obtain cDNA clone for IRBP:
   a) Screen λgt11 library with anti-IRBP antibodies;
   b) Screen λgt10, λgt11 libraries with oligonucleotide probes;
   c) Screen λgt10, λgt11 libraries by counter screening with brain mRNA, with small size mRNAs from retina versus screening with very large mRNAs (>4Kb) from retina. IRBP mRNA is expected to be >4 Kb in length.
2. Sequence the cDNA to derive the amino acid sequence from the mRNA sequence.
3. Screen genomic libraries for human and bovine IRBP genes with the cloned cDNA as probe.
4. Screen cDNA libraries for human IRBP cDNA clones.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 EY 00070-07 LVR

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Vitamin A and Ocular Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR, NEI
Others:	Ling Lee	M.S.	Chemist	LVR, NEI
	Michael Redmond	Ph.D.	Fogarty Fellow	LVR, NEI
	Gerald J. Chader	Ph.D.	Chief	LVR, NEI

COOPERATING UNITS *(if any)*
Division of Biochemistry and Biophysics, Office of Biologics Research and Review, FDA (F. Robey, N. Nguyen); Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH (M. Lewis)

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal Metabolism

INSTITUTE AND LOCATION
NEI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interphotoreceptor Retinoid-Binding Protein (IRBP) was purified to apparent homogeneity from macaque monkey and bovine interphotoreceptor matrix (IPM). The amino acid compositions of the monkey and bovine proteins were similar, with non-polar amino acids comprising more than 50 percent of the residues. Amino terminal analysis showed considerable homology between monkey and bovine IRBPs in this region. Monkey IRBP had an apparent molecular weight of 106,000 ± 2900 on sedimentation equilibrium ultracentrifugation. Both monkey and bovine IRBPs had isoelectric points between 6 and 7. Immunofluorescence studies using affinity purified rabbit anti-monkey IRBP demonstrated the presence of IRBP in Y-79 human retinoblastoma cells grown in monolayer tissue culture. The secretion of IRBP by monkey retina in short-term organ culture was blocked by monensin but was not affected by tunicamiycin or swainsonine.

PHS 6040 (Rev 1/84) GPO 904-917

Project Description:

Additional Personnel Engaged on Project: None

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues as mediated by specific retinoid-binding proteins and to study retinol transport between retinal compartments.

Methods Employed: Affinity chromatography, gel filtration, ion-exchange and size-exclusion high performance liquid chromatography (HPLC), SDS-polyacrylamide gel electrophoresis, sedimentation equilibrium ultracentrifugation, isoelectric focusing, fluorescence spectroscopy, amino-terminal analysis, fluorography, ELISA and immunofluorescence were employed in studying retinoid-binding proteins.

Major Findings: Interphotoreceptor Retinoid-Binding Protein (IRBP) was purified to apparent homogeneity as assessed by silver-stained SDS-polyacrylamide gels from macaque monkey interphotoreceptor matrix (IPM) using affinity chromatography on concanavalin A sepharose followed by ion-exchange and size-exclusion high performance liquid chromatography (HPLC). Bovine IRBP was purified using the same procedure. The amino acid compositions of the monkey and bovine proteins were similar, with non-polar amino acids comprising more than 50 percent of the residues. Amino terminal analysis showed considerable homology between monkey and bovine IRBPs in this region and also verified the purity of the isolated proteins. Sedimentation equilibrium ultracentrifugation of monkey IRBP gave an apparent molecular weight value of 106,000 ± 2900. Preliminary carbohydrate analysis indicated the presence of neutral sugar including fucose and sialic acid. Both monkey and bovine IRBP had isoelectric points between 6.0 and 7.0, with the observed microheterogeneity probably being due to the glycoprotein nature of IRBP.

Rabbit antiserum to monkey IRBP was obtained and characterized by an ELISA assay. Immunofluorescence using affinity purified rabbit anti-monkey IRBP demonstrated the presence of IRBP in Y-79 human retinoblastoma cells grown in monolayer tissue culture. ELISA assays of retinal tissue samples as well as immunofluorescence showed that the distribution of IRBP in human and monkey retinas paralleled the distribution of rod photoreceptor cells within the retina.

Studies on the synthesis and secretion of IRBP by monkey retinas in short-term organ culture demonstrated that the secretion of IRBP by monkey retinas into the culture medium was completely blocked by 10^{-5} M monensin. A proton ionophore known to inhibit the secretion of a variety of proteins from different cells at the level of the Golgi complex. There was still a substantial effect of monensin even at a concentration of 10^{-9} M. 10^{-5} M actinomycin-D also blocked the secretion of IRBP. Neither tunicamycin, which inhibits the initial events in glycosylation of asparagine residues, nor swainsonine, which inhibits α-mannosidase, affected the secretion of IRBP even after a 1 hour preincubation at concentrations of 10^{-5} M.

Significance to Biomedical Research and the Program of the Institute: Most tissues of the body, and ocular tissues in particular, are dependent upon vitamin A for normal growth and development. Retinoid-binding proteins, which are thought to be mediators of vitamin A function are no doubt important factors in the maintenance of normal function in tissues which require vitamin A. Interphotoreceptor Retinoid-Binding protein (IRBP) is a likely candidate as a transport protein for retinoids between retinal compartments, a function which is essential to normal vision.

Studies aimed at elucidating the characteristics and function of IRBP and other retinoid-binding proteins should contribute substantially to the achievement of a better understanding of ocular diseases involving vitamin A metabolism.

Proposed Course: Studies will be continued on the role of Interphotoreceptor Retinoid-Binding Protein (IRBP) in the transport of retinoids between retinal compartments. ELISA assays and immunohistochemistry will be used to compare normal and diseased retinal tissues. The role or roles of cellular retinoid-binding protein in mediating vitamin A function in ocular tissues will also be investigated.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Wiggert B, Masterson E, and Coulombre AJ: Changes in retinoid-binding levels during development of the chicken cornea. Exp Eye Res 37:499, 1983.

Lee L and Wiggert B: Isolation and characterization of an unsaturated fatty acid-binding protein from developing chick neural retina. J Neurochem 42:47, 1984.

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Pfeffer B, Wiggert B, Lee L, Zonnenberg B, Newsome D, and Chader G: The presence of a soluble retinol-binding protein (IRBP) in the retinal interphotoreceptor space. J Cell Physiol 117:333, 1983.

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Rodrigues M, Ballintine E, Wiggert B, Lee L, Fletcher RT, and Chader GJ: Choroideremia: A clinical, electronmicroscopic, and biochemical report. Ophthalmology 91:873, 1984.

Redmond TM, Wiggert B, Lewis M, Robey FA, and Chader GJ: Purification and characterization of monkey interphotoreceptor retinoid-binding protein. Invest Ophthalmol Vis Sci 25(Suppl):274, 1984.

Wiggert B, Lee L, O'Brien PF, and Chader GJ: Synthesis of Interphotoreceptor Retinoid-Binding Protein (IRBP) by monkey retina in organ culture: Effect of monensin. Invest Ophthalmol Vis Sci 25(Suppl):274, 1984.

Chader GJ and Wiggert B: Interphotoreceptor retinoid-binding protein: Characteristics in bovine and monkey retina. Vis Res (in press).

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